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Plasminogen-receptor $_{KT}$: Plasminogen activation and beyond

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Abstract

The cell surface orchestrates plasminogen activation through the concomitant binding of plasminogen and plasminogen activators to specific receptors. In this issue, Miles and colleagues describe their detailed phenotypic characterization of mice deficient in Plg-R_{KT}, a key plasminogen receptor expressed in numerous tissues, but highly expressed by proinflammatory macrophages. The analysis provides critical and surprising new insights into the biology of this receptor.

The plasminogen activation system is a versatile proteolytic system with essential functions in thrombolysis, extravascular fibrin surveillance, suppression of fibrin-associated inflammation, tissue remodeling, tissue regeneration, and more. In addition to these physiological functions, deregulation of the plasminogen activation system is linked to the genesis, progression, or morbidity of a wide variety of important human diseases, including bacterial infection, cancer, neurodegenerative disorders, fibrosis, muscular dystrophy, and rheumatoid arthritis [1-31].

Plasmin, the key effector of most functions of the plasminogen activation system, is a multi-domain trypsin-like serine protease consisting of a pan-apple domain, five kringle domains, and a serine protease domain. It is formed by proteolytic conversion of the catalytically-inactive protease zymogen, plasminogen, by an endoproteolytic cleavage within the activation site of the serine protease domain. Plasminogen is predominantly synthesized by the liver and is present in remarkably high concentrations (1-2 μM) in plasma and in other extravascular fluids [32, 33]. Plasminogen is converted to plasmin either by tissue plasminogen activator (tPA) or by urokinase plasminogen activator (uPA), which are two closely related trypsin-like serine proteases that typically are synthesized, activated, and/or released after disruption of tissue homeostasis, leading to spatially and temporally restricted

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plasmin generation [3]. Once generated, plasmin is inhibited primarily by the fast-acting and abundant serpin-type protease inhibitor, α2-antiplasmin, [34, 35], while tPA- and uPA-mediated activation of plasminogen mainly is inhibited by the serpin-type protease inhibitor, plasminogen activator inhibitor-1 (PAI-1) [36, 37]. Following inhibition by their cognate inhibitors, plasmin and plasminogen activators are internalized by members of the low-density lipoprotein receptor family for lysosomal degradation [38, 39].

Although tPA and uPA both can activate plasminogen in solution, the activation is inefficient, and the newly generated plasmin is susceptible to rapid inhibition by α2-antiplasmin. Rather, the molecular pathways that mediate the conversion of plasminogen to plasmin under physiological conditions involves the formation of ternary complexes between plasminogen and plasminogen activator, with the fibrin polymer or the surface of cells serving as the two principal sites for plasminogen activation. Fibrin strongly promotes activation of plasminogen by tPA by serving as a scaffold for the binding of tPA and plasminogen in a manner that brings the two molecules in close apposition and simultaneously protects the newly generated plasmin from inactivation by α2-antiplasmin [40-43]. Both tPA and uPA mediate cell surface plasminogen activation through the binding to specific cellular receptors that may be constitutively expressed, or induced in response to disruption of tissue homeostasis.

Although cell surface binding has long been recognized to be critical to both the conversion of plasminogen to plasmin and for the subsequent physiological functions of plasmin, the identification and validation of specific cell surface receptors for plasminogen has proved to be a remarkably complex task and the subject of extensive and long-standing investigation (reviewed in [44]). Two factors seem to have contributed to this: The first is the peculiar ability of plasminogen to bind to proteins containing a C-terminal lysine residue via its kringle domains [45]. That this mode of binding is indeed employed by plasminogen during its activation on the cell surface was revealed in early studies, showing that treatment of cells with carboxypeptidase B, which removes C-terminal lysines from proteins, largely abolished the potentiation of plasminogen activation by cells [46]. However, it follows that a large number of cell surface-exposed proteins with C-terminal lysine residues will be amenable to plasminogen binding, although this binding may not be productive in terms of stimulating plasminogen activation or affording protection from α2-antiplasmin. The second factor is the unusually high concentration of plasminogen (1-2 μM) in plasma and interstitial fluids, which means that even cell surface proteins with relatively low affinity for plasminogen must be considered candidate receptors for productive plasminogen activation.

The list of candidate receptors for plasminogen reported thus far is exhaustive, and includes the membrane-associated proteins S100A10 (in complex with annexin A2 within the annexin A2 heterotetramer) [47] and Plg-R_{KT} (see below), as well as, surprisingly, proteins with a normally intracellular location and function including cytoplasmic proteins (α-enolase [46], cytokeratin 8 [48], actin [49]) and nuclear proteins (TIP49a [50] and histone H2B [51]). A subset of integrins have also been identified as plasminogen receptors, including $\alpha_V\beta_3$, $\alpha_M\beta_2$, and $\alpha_{IIb}\beta_3$. The role of these integrins as plasminogen receptors is notable in that these receptors do not engage plasminogen through a C-terminal lysine and do not significantly enhance plasminogen activation (reviewed in [44]).

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Originally identified from a membrane proteome screen of differentiated mouse macrophages, the 147- amino acid Plg-R_{KT} is unique compared to previously described plasminogen receptors, as it is present exclusively on the cell surface, being synthesized as an integral membrane protein that supports plasminogen binding through a C-terminal lysine. Plg-R_{KT} is highly conserved across mammalian species with homologs also in *Xenopus*, *Drosophila*, and zebrafish, and, importantly, all of the mammalian orthologs of Plg-R_{KT} contain a C-terminal lysine residue [52]. Like many of the proposed plasminogen receptors, Plg-R_{KT} is broadly expressed in mammalian tissues and notably in many hematopoietic-derived cells. Plg-R_{KT} also significantly enhances plasminogen activation by supporting binding of plasminogen activators. tPA binds Plg-R_{KT} through the same C-terminal lysine domain and, thus, can enhance plasmin generation through plasminogen bound to an adjacent Plg-R_{KT} molecule. In addition, Plg-R_{KT} is clustered on the cell surface with uPA when bound to its receptor uPAR. This colocalization of plasminogen activators and plasminogen has been proposed as a key mechanism by which Plg-R_{KT} regulates cell surface associated plasmin generation [44, 52, 53].

The broad expression pattern of plasminogen receptors in general, and Plg-R_{KT} in particular, coincides with the numerous physiologic and pathophysiologic processes in which cell-surface associated plasmin generation has been proposed to participate (*i.e.,* thrombus resolution, inflammation, bacterial infection, wound healing, neuronal function, tumor progression, metastasis, muscle injury and repair, and bone homeostasis). However, the numerous identified candidates, as well as the overlapping cellular expression pattern of these proteins, complicate defining the precise roles of specific plasminogen receptors in various processes. In order to address this concept and provide a valuable new tool for *in vivo* analysis of the PA system, Miles and colleagues recently generated Plg-R_{KT} knockout (Plg-$R_{KT}^{-/-}$) mice through a standard homologous recombination strategy in mouse embryonic stem cells (see pages XXX of this issue of JTH). The baseline characterization of these animals supports key roles for plasmin(ogen) that are linked to Plg-R_{KT} binding and plasmin functions that are independent of Plg-R_{KT}. Furthermore, unexpected phenotypes suggest novel functions for Plg-R_{KT} independent of plasminogen itself.

Like plasminogen knockout ($Plg^{-/-}$) mice, Plg-$R_{KT}^{-/-}$ mice are viable and fertile. Although Plg-$R_{KT}^{-/-}$ female mice can carry a litter to term, they are incapable of supporting even an initial litter of neonates to weaning. This failure of pup survival was linked to a severe lactation defect where milk production in Plg-$R_{KT}^{-/-}$ females was severely decreased within 2 days postpartum. $Plg^{-/-}$ mice also have a documented impairment in lactational competence, however it is markedly less severe. Indeed, many $Plg^{-/-}$ females can support a first litter to weaning, but routinely fail to support a second litter indicating a significant deleterious event following the first mammary gland involution episode. The mammary gland defect observed in $Plg^{-/-}$ mice was linked to persistent fibrin accumulation [31, 54]. The fact that the phenotype in Plg-$R_{KT}^{-/-}$ mice was more severe than that observed for $Plg^{-/-}$ mice suggests a plasminogen-independent mechanism. It is possible that Plg-R^{KT} serves as a receptor for a second, as yet unidentified, protease that functions to modify the ECM during mammary gland development. Alternatively, it is possible that Plg-R_{KT} works in concert with other cell surface or integral membrane proteins to support mammary gland development. To this end, it is notable that mammary epithelial cell deficiencies in either β1

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integrin or the gap junction protein connexin 43 have similar mammary gland defects with diminished milk production as that described for Plg-$R_{KT}^{-/-}$ mice [55, 56]. The precise cellular and molecular basis of lactation incompetence in Plg-$R_{KT}^{-/-}$ remains to be established.

Perhaps the most striking spontaneous phenotype for $Plg^{-/-}$ mice is a severe wasting disease leading to early mortality experienced by both male and female mice. Survival analysis of Plg-$R_{KT}^{-/-}$ mice indicated survival patterns similar to wildtype mice and body wasting was not observed, at least for male mice. Female Plg-$R_{KT}^{-/-}$ did show a progressive reduction in weight gain over time starting at 4.5 weeks of age, however the mechanistic basis for this gender-specific diminution in growth rate remains undefined. The severe wasting disease and early mortality characteristic of $Plg^{-/-}$ mice is mechanistically linked to multi-organ, persistent fibrin accumulation, as superimposing fibrinogen-deficiency on $Plg^{-/-}$ mice rescued both the progressive weight loss and early mortality [15]. A multi-organ histological survey revealed no evidence of extravascular fibrin deposits in Plg-$R_{KT}^{-/-}$ mice, and Plg-$R_{KT}^{-/-}$ mice did not display other related phenotypes typically observed in $Plg^{-/-}$ mice (*e.g.,* rectal prolapse, ligneous conjunctivitis) [5, 14-16]. Interestingly, mice deficient in either annexin A2 or S100A10 have increased microvascular fibrin deposition in multiple organs suggesting that, unlike Plg-R_{KT}, the annexin A2-S100A10 plasminogen receptor plays a key role in baseline plasmin-mediated fibrin surveillance and clearance [57]. Annexin A2-S100A10-deficient mice also display a compromised ability to clear arterial thrombi following injury [58, 59]. Whether or not Plg-R_{KT} plays any role in thrombus clearance, be it the clearance of arterial thrombi or venous thrombi, remains to be established. However, Plg-$R_{KT}^{-/-}$ mice provide an ideal tool for addressing these very questions in an *in vivo* model system.

The initial identification of Plg-R_{KT} from macrophages implicated the receptor in macrophage function and inflammation. Indeed, Plg-$R_{KT}^{-/-}$ mice were shown to have an ~80% reduction in macrophage recruitment to the peritoneal cavity of mice using the thioglycollate model. This finding was consistent with previous studies showing that systemic administration of an antibody against Plg-R_{KT} reduced macrophage trafficking to the peritoneal cavity following thioglycollate injection by 49% [53]. Modifying inflammation and macrophage activity appears to be a point of commonality for many of the plasminogen receptors, as multiple plasminogen receptors, including annexin A2-S100A10, enolase-1, histone 2B, TATA-binding protein interacting protein, $\alpha_M\beta_2$, and Plg-R_{KT}, are expressed on the cell surface of monocytoid cells. Accordingly, targeting other individual plasminogen receptors similarly reduced macrophage migration in the mouse thioglycollate model. Antibodies directed against histone 2B and enolase-1 significantly reduced macrophage accumulation by 48% and 24%, respectively [60]. Additionally, $S100A10^{-/-}$ mice display 53% less macrophage recruitment following thioglycollate stimulation [59]. Each of these findings aligned well with results of thioglycollate challenge in $Plg^{-/-}$ mice. A 65% reduction in macrophage recruitment following thioglycollate challenge in $Plg^{-/-}$ mice compared to wildtype mice was observed [61]. That elimination or blockade of any one plasminogen receptor on macrophages results in a significant diminution of migration following an identical chemotactic stimulus is intriguing and suggests one of two possibilities: (*i*) individual plasminogen receptors may be working coordinately as part of a

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complex, such that loss of any one member significantly impacts the functionality of all or (*ii*) individual receptors may support different critical aspects of the migratory process with each step requiring plasminogen. Indeed, a more in depth characterization of the migratory process with respect to the role of plasminogen and receptors, including the impact of combinatorial loss of receptors is warranted.

Beyond simple studies of macrophage migration, the broader role of Plg-R_{KT} and other plasminogen receptors in inflammation and inflammatory disease remains an open question. Inflammatory stimuli increase surface expression of several plasminogen receptors (annexin A2-S100A10, enolase-1, histone 2B, and Plg-R_{KT}) and multiple plasminogen receptors (annexin A2, enolase-1, histone 2B) are targets of autoantibody production in the context of autoimmune diseases [62-65]. Plg-$R_{KT}^{-/-}$ mice do not appear to be particularly susceptible to spontaneous infectious or inflammatory events. Note that a modest increase in dermatitis was reported for Plg-$R_{KT}^{-/-}$ mice but strain C57Bl/6 animals are inherently susceptible to dermatitis [66]. However, perhaps a more interesting open question is to understand the contribution of cell surface-associated plasmin activity and Plg-R_{KT} to specific inflammatory diseases, particularly those where macrophages play a preeminent role in pathogenesis (*e.g.,* microglial cells and neuroinflammatory disease, Kupffer cells and hepatotoxic injury, M1-type adipose tissue macrophages and obesity). Indeed, given that extravascular fibrin deposits are a near universal feature of inflammatory foci, it will be interesting to determine whether plasmin-Plg-R_{KT} plays a role in clearing inflammation-associated pathological fibrin deposits or whether Plg-R_{KT}-restricted plasmin activity functions in inflammation through fibrin-independent mechanisms. Further, the PA system and macrophages each are implicated in tumor progression. Plg-R_{KT} is upregulated in many tumor types and may play a role in this disease. It is also possible that Plg-R_{KT} may function in cancer through a mechanism linked to M2-type tumor promoting macrophages. Such possibilities highlight both the complexity of the PA system in disease and the need for more in-depth analyses.

In conclusion, Plg-$R_{KT}^{-/-}$ mice provide a valuable new reagent for understanding the contribution of cell surface-associated plasminogen activation in physiological and pathological processes. These mice should help provide clarity to the expanding and complicated field of plasminogen receptor biology by better defining roles for the most unique member of this receptor family. Further, these mice have the capacity to expand our knowledge of basic cell biology, as the current data suggests Plg-R_{KT} may serve vital biological roles through mechanisms independent from plasminogen binding and activation. That Plg-$R_{KT}^{-/-}$ mice are viable and largely phenotype-free in the absence of a specific challenge suggests that pharmacological targeting of Plg-R_{KT} in a pathological context would be a viable and attractive novel therapeutic strategy.

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