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REVIEW ARTICLE

Functions of the plasminogen receptor Plg-R_{KT}

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Abstract

Plg-R_{KT} is a structurally unique transmembrane plasminogen receptor with both N- and C-terminal domains exposed on the extracellular face of the cell. Its C-terminal lysine functions to tether plasminogen to cell surfaces. Overexpression of Plg-R_{KT} increases cell surface plasminogen binding capacity while genetic deletion of Plg-R_{KT} decreases plasminogen binding. Plasminogen binding to Plg-R_{KT} results in promotion of plasminogen activation to the broad spectrum serine protease plasmin. This function is promoted by the physical association of Plg-R_{KT} with the urokinase receptor (uPAR). Plg-R_{KT} is broadly expressed in cells and tissues throughout the organism and its sequence is remarkably conserved phylogenetically. Plg-R_{KT} also is required for lactation and, thus, is necessary for survival of the species. This review provides an overview of established and emerging functions of Plg-R_{KT} and highlights major roles for Plg-R_{KT} in both the initiation and resolution of inflammation. While the roles for Plg-R_{KT} in the inflammatory response are predominantly plasmin(ogen)-dependent, its role in lactation requires both plasminogen-dependent and plasminogen-independent mechanisms. Furthermore, the functions of Plg-R_{KT} are dependent on sex. In view of the broad tissue distribution of Plg-R_{KT}, its role in a broad array of physiological and pathological processes should provide a fruitful area for future investigation.

KEYWORDS

fibrin, fibrinolysis, plasminogen, PLG-R(KT) protein, receptors

1 | INTRODUCTION

This review article focuses on functions of the novel plasminogen receptor Plg-R_{KT}. Here we briefly summarize general concepts that apply to the interaction of plasminogen with cells, followed by a summary of unique aspects of Plg-R_{KT} sequence and topology and its in vitro function. We then discuss, in depth, recent data implicating a major role of Plg-R_{KT} in innate immune function as a plasminogen receptor as well as surprising data revealing an unexpected role of Plg-R_{KT} in lactation in which Plg-R_{KT} exhibits both plasminogen-dependent and plasminogen-independent roles. We discuss how sex as a biological variable affects Plg-R_{KT} function. Finally, we summarize emerging functional roles of Plg-R_{KT} in pathophysiological processes.

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2 | KEY FEATURES OF THE INTERACTION OF PLASMINOGEN WITH CELLS

2.1 | Mechanisms for promoting association of plasmin activity with cell surfaces

The ability of plasminogen to specifically bind to cell surfaces was first demonstrated on platelets.[1] Subsequent studies have shown that plasminogen binding sites are present almost ubiquitously on eukaryotic cells and in tissues as well as on many prokaryotic cells (reviewed in Miles and Parmer[2]). Localization of plasminogen on cell surfaces promotes its activation to the broad spectrum serine protease, plasmin, in a reaction mechanism that is promoted when plasminogen activators (tissue plasminogen activator [t-PA] or urokinase [u-PA]) are colocalized with plasminogen (reviewed in Miles et al[3]), analogous to plasminogen activation on fibrin to promote fibrinolysis.[4] In addition, plasminogen activation is promoted when the interaction of Glu-plasminogen (the native, circulating form of plasminogen) with cells induces a conformation exposing the cleavage site for plasmic removal of its N-terminal 77 amino acids[5] to form the more readily activatable Lys-plasminogen.[6-8] As an additional mechanistic consideration, enhancement of plasminogen activation on eukaryotic cell surfaces is lost following treatment of cells with carboxypeptidase B (CpB).[9] CpB proteolytically removes C-terminal basic residues. Hence, plasminogen binding proteins exposing C-terminal basic residues on cell surfaces are predominantly responsible for stimulation of plasminogen activation. Furthermore, plasmin formed on the cell surface is retained on the cell membrane and is protected from inactivation by its major inhibitor, α_2-antiplasmin,[10,11] most likely due to competition between cell surface C-terminal basic residues with the C-terminal lysine of α_2-antiplasmin that is required for the initial interaction with plasmin.[12]

2.2 | Physiological and pathophysiological roles of cell-associated plasmin

Localization of the broad spectrum proteolytic activity of plasmin on cells has important consequences for physiological and pathological processes, many of which require fibrin proteolysis as well as degradation of extracellular matrix (ECM) components to allow cells to migrate, including the inflammatory response, wound healing, oncogenesis, metastasis, myogenesis and muscle regeneration (reviewed in Miles et al[3]). Prohormone processing, neurite outgrowth, and fibrinolysis are also regulated by cell-associated plasmin (reviewed in Miles et al[3]). Cell-bound plasmin also stimulates cell signaling pathways to induce release of cytokines, reactive oxygen species, and other inflammatory mediators.[13,14]

2.3 | Cell surface plasminogen binding proteins

The diversity of cell types that bind plasminogen as well as the high capacity of cells for plasminogen (reviewed in Miles and Plow[1] and Miles et al[15]) has led to identification of numerous plasminogen receptors. Prior to the discovery of Plg-R_{KT}, CpB-sensitive cellular plasminogen receptors comprised two groups: (a) proteins synthesized with C-terminal basic residues and having well recognized intracellular functions, including α-enolase,[16,17] cytokeratin 8,[18,19] S100A10 (in complex with annexin A2 within the annexin A2 heterotetramer),[20] TIP49a,[21] and histone H2B[22] and; (b) proteins that undergo proteolytic processing in order to expose a C-terminal basic residue to permit plasminogen binding, including actin.[23-25] There is also a CpB-insensitive component of plasminogen binding to eukaryotic cells that does not promote plasminogen activation,[9] which includes tissue factor[26] and non-proteinaceous gangliosides.[27] Several transmembrane proteins ($\alpha IIb\beta_3$,[28] $\alpha_M\beta_2$,[29,30] and $\alpha_5\beta_1$,[30] as well as amphoterin[31] and GP330[32]) bind plasminogen but are not synthesized with C-terminal basic residue and it has not been established whether these proteins undergo proteolysis to reveal C-terminal basic residues. Detailed reviews on the identification, functions, and mechanisms of cell surface-association of the plasminogen receptors discussed above can be found in the volume introduced by Miles et al.[33]

3 | DISCOVERY OF Plg-R_{KT}

3.1 | Proteomic discovery of the novel plasminogen receptor, Plg-R_{KT}

In the absence of identification of an integral membrane protein exposing a carboxyl terminal basic residue on the cell surface, a proteomic approach was applied as a sensitive technique that could potentially reveal such a protein. Cell surface labeling of an inducible monocytoid stem cell line, followed by targeted proteolysis with CpB, affinity chromatography on plasminogen and streptavidin, and multidimensional protein identification technology (MudPIT) was performed.[3,34] One protein with a predicted transmembrane sequence and a C-terminal basic residue was identified: the hypothetical protein, C9orf46 homolog (IPI00136293), homologous to the protein predicted to be encoded by human chromosome 9, open reading frame 46.[34] Interestingly, the MudPIT analysis also detected peptides from other proteins previously characterized as plasminogen receptors on monocytes: α-enolase, histone H2B, gamma actin, S100A10, annexin A2, and β2 integrin, as validation of the method.

3.2 | Characteristics of Plg-R_{KT}

The C9orf46 homolog murine DNA sequence encodes a protein of 147 amino acids with a predicted molecular mass of 17 261 Da and a C-terminal lysine (Figure 1A). The protein was named Plg-R_{KT}, to indicate a plasminogen receptor with a C-terminal lysine and a transmembrane domain. The sequences of more than 65 mammalian orthologs are now available and alignment of these sequences shows high identity (eg, human versus chimpanzee = 99% identity; human versus grizzly bear = 87.8% identity) with no gaps in

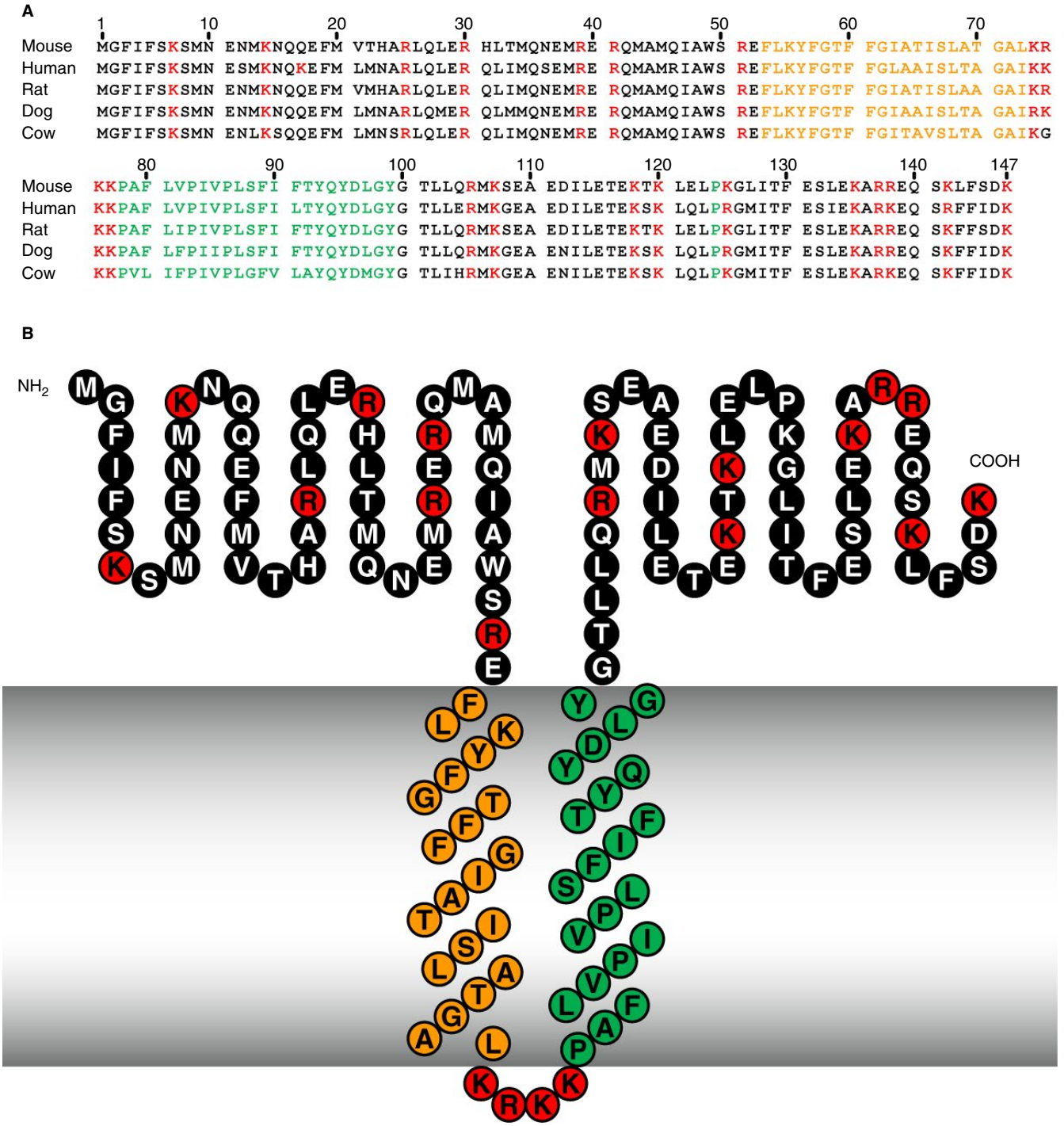

FIGURE 1 Alignment of predicted amino acid sequences of mouse, human, rat, dog, and cow orthologs of Plg-R_{KT} (A) and the structural model of Plg-R_{KT} (B) show high interspecies sequence homology. Green indicates amino acids within the predicted primary transmembrane helix. Orange indicates amino acids within the predicted secondary transmembrane helix. Red indicates basic amino acids. This research was originally published in *Blood*, Andronicos et al.[34] © the American Society of Hematology

the sequences. Importantly, a C-terminal lysine is encoded for all sequenced mammalian orthologs (eg, Miles et al[35]). Furthermore, DNA sequences of phylogenetically lower species (green lizard, xenopus, and zebrafish) encode C-terminal lysines. Also contained within the Plg-R_{KT} sequence is a conserved DUF2368 domain (spanning amino acids 1-135), an uncharacterized protein with unknown function that is evolutionarily conserved from nematodes to humans. The Plg-R_{KT} orthologs of lower organisms (eg, Drosophila, Paramecium, and the sea urchin Strongylocentrotus purpuratus) predict proteins of different lengths and do not always predict C-terminal lysines. Notably, plasminogen is considered to have evolved evolutionarily with protochordates.[36] Thus, organisms evolutionarily below protochordates might not need the C-terminal lysine of Plg-R_{KT}.

Predictive websites indicate a preferred topology model with two transmembrane helices extending from F_{53}-L_{73} (oriented from outside the cell to inside the cell) and P_{78}-Y_{99} (oriented from inside the cell to outside the cell) with a short 4 basic amino acid cytoplasmic sequence (Figure 1B).[34] Thus, a 52 amino acid N-terminal region and a 48 amino acid C-terminal tail with a C-terminal lysine are predicted to be exposed on the cell surface. This model is supported by several lines of evidence: (a) in phase separation experiments Plg-R_{KT} partitions to the detergent phase, consistent with integral membrane protein behavior;[34,37] (b) protease accessibility experiments support extracellular exposure of both N- and C-termini of Plg-R_{KT};[35,38] (c) peptides corresponding to Plg-R_{KT} are not recovered when intact cells are treated with CpB, prior to proteomic analysis, in accordance with cell surface exposure of the C-terminus of Plg-R_{KT};[34] and (d) a monoclonal antibody (mAB) raised against the C-terminal peptide of Plg-R_{KT} reacts with the cell surface.[34,37,39]

Plg-R_{KT} exhibits key requisite attributes of a plasminogen receptor. Plasminogen binds directly to the C-terminal peptide of Plg-R_{KT} and a mAB raised against this region reacts with plasminogen binding sites on cells.[34] Overexpression of Plg-R_{KT} increases cell surface plasminogen binding capacity.[37] Genetic deletion of Plg-R_{KT} markedly decreases plasminogen binding to cells.[39] Plg-R_{KT} promotes plasminogen activation by both t-PA[34] and u-PA.[40] Plg-R_{KT} colocalizes[34,39] and co-immunoprecipitates[37] with the urokinase receptor (uPAR) as a mechanism to promote plasminogen activation. Notably, t-PA binds specifically to a peptide corresponding to the C-terminus of Plg-R_{KT}.[34] Despite the potential to compete for binding to Plg-R_{KT}, the relative concentrations of circulating plasminogen and t-PA should permit simultaneous binding of both ligands to distinct Plg-R_{KT} molecules so that each t-PA molecule should be bound proximally to several plasminogen molecules to promote plasminogen activation by t-PA.[41]

Plg-R_{KT} has a broad tissue distribution, as determined by probing human and mouse tissue microarrays with a panspecific monoclonal antibody[40] raised against a synthetic nonapeptide corresponding to the C-terminus of rat Plg-R_{KT}. Plg-R_{KT} is expressed in murine and human tissues including neuroendocrine tissue (thyroid and adrenal gland); brain; spleen; lymph nodes; vasculature; and very prominently, in epithelial tissues including kidney, breast, lung, intestine, and colon.[42] In mammary tissue, high Plg-R_{KT} expression is present in epithelial tissue of tubuloalveolar glands and lactiferous ducts.[42,43] In lungs, Plg-R_{KT} expression is notable on pseudostratified and simple columnar epithelium in bronchi. And absorptive epithelium in small intestine and colon exhibit high Plg-R_{KT} expression.[42] By western blotting, high expression of Plg-R_{KT} is detected in human lymphocytes, peripheral blood monocytes, and endothelial cells, with much lower expression in neutrophils, while not detectable in red blood cells (RBC).[40] The broad distribution in tissues that are exposed to plasminogen suggests that Plg-R_{KT} may regulate proteolytic activity throughout the organism.

4 | ROLE OF Plg-R_{KT} IN INNATE IMMUNITY

In response to infectious or sterile injurious stimuli, an inflammatory response is initiated leading to increased vascular permeability, release of pro-inflammatory mediators, and subsequent leukocyte recruitment.[44] Subsequently, anti-inflammatory mechanisms come into play, followed by the resolution of inflammation, an active process that functions to restore tissue homeostasis.[45] Plg-R_{KT} exerts a major impact on each of these processes as discussed below and as summarized in Figure 2.

4.1 | Regulation of leukocyte recruitment by Plg-R_{KT}

Studies in plasminogen-deficient mice have revealed a key role for plasminogen in macrophage and lymphocyte recruitment during the inflammatory response.[46-49] Previous studies indicate that active plasmin is required to promote leukocyte recruitment[49] and plasminogen-dependent macrophage recruitment is mediated by CpB-sensitive plasminogen binding sites.[50] Consistent with these requirements, injection of mice with anti-Plg-R_{KT} mAB impairs mononuclear leukocyte recruitment in a model of sterile peritonitis induced by injection of thioglycollate. (Treatment with thioglycollate results in sequential recruitment of neutrophils, monocytes, and lymphocytes to the peritoneum.) Treatment with anti-Plg-R_{KT} mAB inhibits both macrophage recruitment (by 53%) and lymphocyte recruitment (by 60%) without affecting either neutrophil or eosinophil recruitment.[40] Macrophage and lymphocyte recruitment are also impaired in this model in plasminogen-deficient mice, without an effect on neutrophils.[49,51] Similar effects on selective leukocyte recruitment occur in response to pleural injection of plasmin[52,53] or lipopolysaccharide (LPS).[54] The absence of a genotype effect on neutrophil recruitment is consistent with relatively low expression of Plg-R_{KT} on neutrophils[40] and relatively low plasminogen binding capacity of neutrophils.[55] When anti-Plg-R_{KT} mAB is injected into plasminogen-deficient mice, there is no further decrease in macrophage recruitment, illustrating that Plg-R_{KT} functions to bind plasmin(ogen) in the thioglycollate model[40] (Figure 3).

Effects of genetic deletion of Plg-R_{KT} on macrophage recruitment have been demonstrated in two models of inflammation. In time course experiments there is no effect of Plg-R_{KT} deletion until 72 hours following thioglycollate injection when total leukocyte recruitment is significantly impaired and macrophage recruitment is 76% lower in Plg-$R_{KT}^{-/-}$ mice, compared with Plg-$R_{KT}^{+/+}$ littermate controls.[56] In the second model, stimulation of inflammation by intrapleural injection of LPS results in time-dependent recruitment of leukocytes into the pleural cavity with early neutrophilic recruitment and resolution at 48 hours when neutrophils are scarce and mononuclear cell infiltration is maximal.[57,58] In this model of pleurisy, recruitment of mononuclear cells and pleural levels of monocyte chemoattractant chemokine, CCL2, are significantly lower in Plg-$R_{KT}^{-/-}$ mice compared to Plg-$R_{KT}^{+/+}$ littermates, with no significant

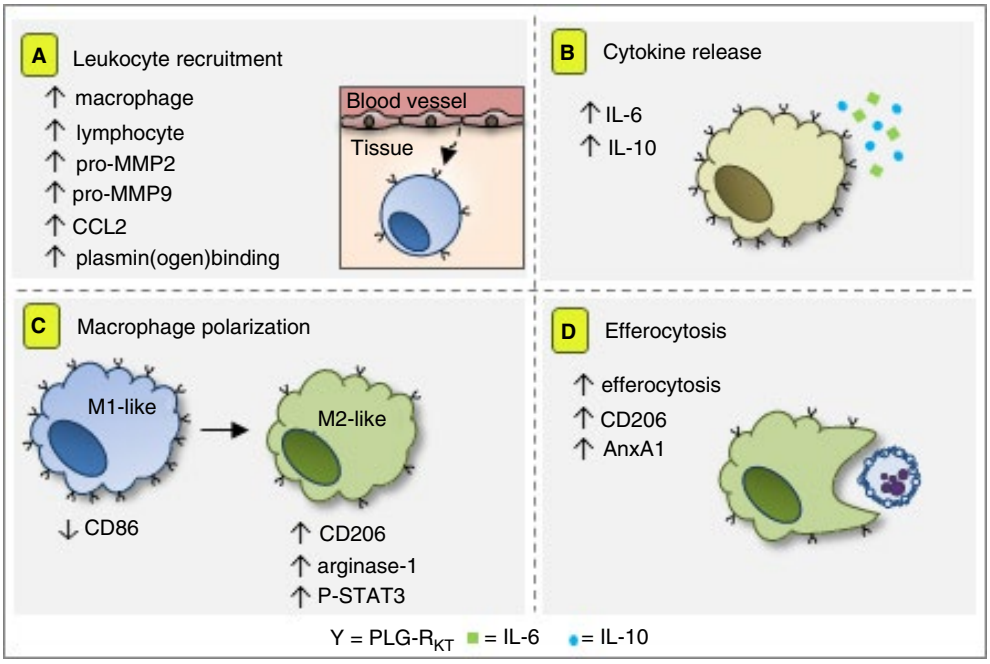

FIGURE 2 Schematic representation of the role of Plg-R_{KT} in innate immunity. Plg-R_{KT} receptor is required for macrophage and lymphocyte recruitment to inflammatory sites. A, Mechanistically, this effect is mediated by plasmin(ogen) binding and activation via Plg-R_{KT}, by the release of the monocyte chemoattractant CCL2 and by activation of the matrix metalloproteinases pro-matrix metalloproteinase-2 (MMP-2) and pro-MMP-9. B, Plg-R_{KT} receptor increases the release of interleukin (IL)-6 and IL-10 cytokines. C, Plg-R_{KT} is preferentially expressed on M1-like macrophages, while during inflammation Plg-R_{KT} decreases CD86 expression. In a similar way, Plg-R_{KT} promotes expression of macrophage M2 markers and (D) enhances efferocytosis of apoptotic cells (4). This diagram has not been published previously.

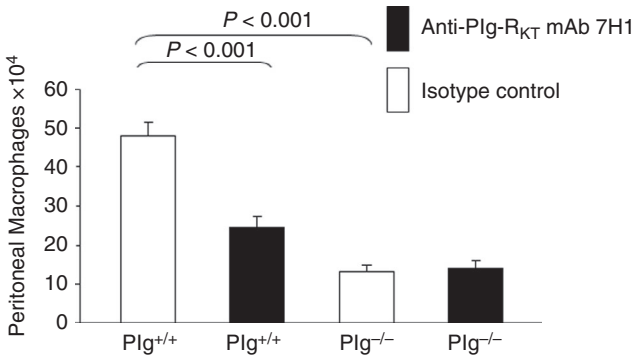

FIGURE 3 Effect of Plg-R_{KT} on thioglycollate-induced monocyte recruitment. Both plasminogen-deficient ($Plg^{-/-}$) and wild type littermates ($Plg^{+/+}$) mice were injected intravenously with either monoclonal antibody 7H1 (■) or isotype control (□) (500 μg). After 30 minutes thioglycollate was injected intraperitoneally. A second injection of antibody was given 24 hours later. After 72 hours, thioglycollate-recruited cells were collected by peritoneal lavage and macrophages were purified by adherence. The adherent cells were detached and counted using a hemocytometer. Data represent mean ± standard error of the mean n = 5/group). This research was originally published in *Blood*, Lighvani et al.[40] © the American Society of Hematology

effect of genotype on recruitment of neutrophils or levels of the neutrophil chemoattractant chemokine CXCL-1.[54] A similar effect is observed in plasminogen-deficient mice,[54] suggesting plasminogen dependence of the functions of Plg-R_{KT} in this model.

4.1.1 | Mechanisms by which Plg-R_{KT} regulates leukocyte recruitment

The in vivo studies summarized above are consistent with Plg-R_{KT} functioning to bind plasminogen and promote plasminogen activation to plasmin on the surfaces of migrating monocytoid cells and lymphocytes. In vitro studies also support the plasminogen binding functions of Plg-R_{KT}. Anti-Plg-R_{KT} mAB blocks plasminogen dependent monocytoid cell migration through Matrigel as well as plasminogen-dependent chemotactic migration in response to the monocyte chemoattractant CCl2 (MCP-1).[40]

Plg-R_{KT} plays a role in the activation of pro-matrix metalloproteinases (MMPs). In the thioglycollate-induced sterile peritonitis model, activation of pro-MMP-9 is required for plasmin(ogen)-dependent macrophage recruitment[49] and activation of pro-MMP-9 and pro-MMP-2 is observed in peritoneal fluid.[40] Injection of anti-Plg-R_{KT} mAB decreases pro-MMP-9 activation by 64% and pro-MMP-2 activation by 44%.[40] In studies in vitro, anti-Plg-R_{KT} mAB blocks activation of pro-MMP-9 and pro-MMP-2 synthesized by monocytoid cells.[40]

It has recently been recognized that Plg-R_{KT} may additionally regulate chemokine ligand 2 (CCL2) levels as a new mechanism for regulation of macrophage migration. In the LPS-induced pleurisy model the lower number of mononuclear cells recruited to the pleural cavity is accompanied by a significantly lower level of CCL2 in the pleural cavity of Plg-$R_{KT}^{-/-}$ mice, compared with Plg-$R_{KT}^{+/+}$ littermates.[54] This is also observed in plasminogen-deficient mice.[54]

Similarly, peritoneal CCL2 levels are decreased in plasminogen-deficient mice following biomaterial implantation.[48] Furthermore, treatment of mice with plasmin(ogen) increases monocyte migration to the pleural cavity via a mechanism dependent on CCL2/chemokine receptor 2 (CCR2).[52] Strikingly, there is no effect of specific deletion of Plg-R_{KT} in myeloid cells (mPlg-$R_{KT}^{-/-}$ mice) on recruitment to the pleural cavity or on pleural CCL2 levels.[54] Thus, Plg-R_{KT} deletion in other cell types may affect synthesis of CCL2 and impact monocyte recruitment in mice with global deletion of Plg-R_{KT}.

Preferential expression of Plg-R_{KT} on pro-inflammatory monocyte and macrophages is a newly recognized mechanism for regulation of their migration and recruitment. Pro-inflammatory human intermediate monocytes ($CD14^{++}CD16^{+}$) express significantly higher levels of Plg-R_{KT} on their surfaces compared with classical monocytes ($CD14^{++}CD16^{-}$) and nonclassical monocytes ($CD14^{+}CD16^{++}$) and this is paralleled by significantly higher plasminogen binding capacity on intermediate monocytes.[39] Mechanistically, intermediate monocytes exhibit plasmin(ogen)-dependent cell migration that is blocked by anti-Plg-R_{KT} mAB.[39] Furthermore, in murine blood, the pro-inflammatory $Ly6C^{high}$ monocyte subset expresses significantly more Plg-R_{KT} and plasminogen binding capacity than the $Ly6C^{low}$ subset and both wild type monocyte subsets bind significantly more plasminogen than Plg-$R_{KT}^{-/-}$ subsets.[39] In the sterile peritonitis model $Ly6C^{high}$ monocyte recruitment is decreased by 57% in Plg-$R_{KT}^{-/-}$ mice compared to Plg-$R_{KT}^{+/+}$ littermates, but there is no genotype effect on recruitment of $Ly6C^{low}$ cells.[39]

4.2 | Role of Plg-R_{KT} in inflammatory cytokine synthesis and release

The ability of plasmin to stimulate intracellular signaling pathways and cytokine release by monocytes and macrophages is well established in vitro and requires an interaction of plasmin(ogen) with the cell surface.[14,59-61] Following thioglycollate stimulation, peritoneal levels of interleukin 6 (IL-6) are increased more than seven-fold in Plg-$R_{KT}^{+/+}$ mice, but < two-fold in Plg-$R_{KT}^{-/-}$ mice and IL-10 levels are three-fold lower in Plg-$R_{KT}^{-/-}$ compared with Plg-$R_{KT}^{+/+}$ mice.[39] (IL-10 has anti-inflammatory properties and is also induced under inflammatory conditions[62,63].)

4.3 | Regulation of resolution of inflammation by Plg-R_{KT}

4.3.1 | Regulation of macrophage polarization by Plg-R_{KT}

During the resolution phase of inflammation, macrophages are reprogrammed to a spectrum of M2-like resolving phenotypes.[64] In the LPS-induced pleurisy model, a higher proportion of M1-like macrophages are present in the pleural exudates of both Plg-$R_{KT}^{-/-}$ and plasminogen-deficient mice, compared with their wild type littermates, suggesting an impairment in polarization to the M2-like phenotype, with reduced expression of the engulfment and recognition molecules, CD206 and Annexin A1.[54] Furthermore, polarization of Plg-$R_{KT}^{-/-}$ and plasminogen-deficient bone marrow derived macrophages (BMDMs) toward an M2-like phenotype (with either IL-4 or IL-10) is impaired, with reduction in the expression of well established M2 markers resulting from IL-4 stimulation (CD206 and arginase-1) and IL-10 polarizing agents (TGFβ).[54] On the other hand, M1-like Plg-$R_{KT}^{-/-}$ BMDMs exhibit increased expression of the M1 marker, CD86.[54] IL-4 can stimulate macrophage polarization through either the STAT6 (canonical) or STAT3 (non-canonical) pathways, while IL-10 triggers the STAT3 pathway.[65] Treatment of both Plg-$R_{KT}^{-/-}$ and plasminogen-deficient BMDMs with IL-4 (Figure 4A,C) or IL-10 (Figure 4B,C) results in a reduction in phosphorylated STAT3 but not phosphorylated STAT6.[54] This suggests a mechanism in which plasmin(ogen) interacts with Plg-R_{KT} to stimulate STAT3 phosphorylation and is the first demonstration of intracellular signalling mediated by this receptor.

Interestingly, proinflammatory M1-like macrophages exhibit significantly more cell surface Plg-R_{KT} than either unpolarized or M2-like macrophages.[39] Thus, Plg-R_{KT} functions to promote polarization and is then down-regulated as a potential negative feedback mechanism.

4.3.2 | Regulation of efferocytosis by Plg-R_{KT}

Following their recruitment to inflammatory sites, and having exerted effector functions, neutrophils undergo apoptosis. An essential step to decrease tissue exposure to toxic contents of dying cells is their phagocytic engulfment by macrophages (efferocytosis).[66] Plasmin(ogen) plays a key role in phagocytosis and this requires its interaction with cell surfaces and protein synthesis.[53,67-70] Efferocytosis in vitro is also significantly decreased in Plg-$R_{KT}^{-/-}$ and plasminogen-deficient BMDMs.[54] When macrophage recruitment is stimulated by intraperitoneal injection of zymosan followed by injection of fluorescently labelled apoptotic neutrophils, engulfment of neutrophils is significantly decreased in Plg-$R_{KT}^{-/-}$, mPlg-$R_{KT}^{-/-}$, and plasminogen-deficient mice relative to their wild type littermate controls.[54] Macrophage expression of the engulfment and recognition molecules CD206 and AnxA1 is significantly lower in these deficient mice.[54] Indeed, the expression of AnxA1, among other sets of genes involved in phagocytosis, is modulated in liver following injection of anti-RBC autoantibody and in spleen in response to injection of apoptotic thymocytes in plasminogen-deficient mice[68] and plasmin(ogen) promotes efferocytosis in an AnxA1-dependent fashion.[58] Thus, binding of plasmin(ogen) to Plg-R_{KT} may promote a mechanism to stimulate AnxA1 expression to further amplify the process of efferocytosis.

5 | SEX AS A BIOLOGICAL VARIABLE IN THE PLASMINOGEN ACTIVATION SYSTEM

Sex has significant effects on the plasminogen activation system. Circulating plasminogen levels in male mice are significantly lower

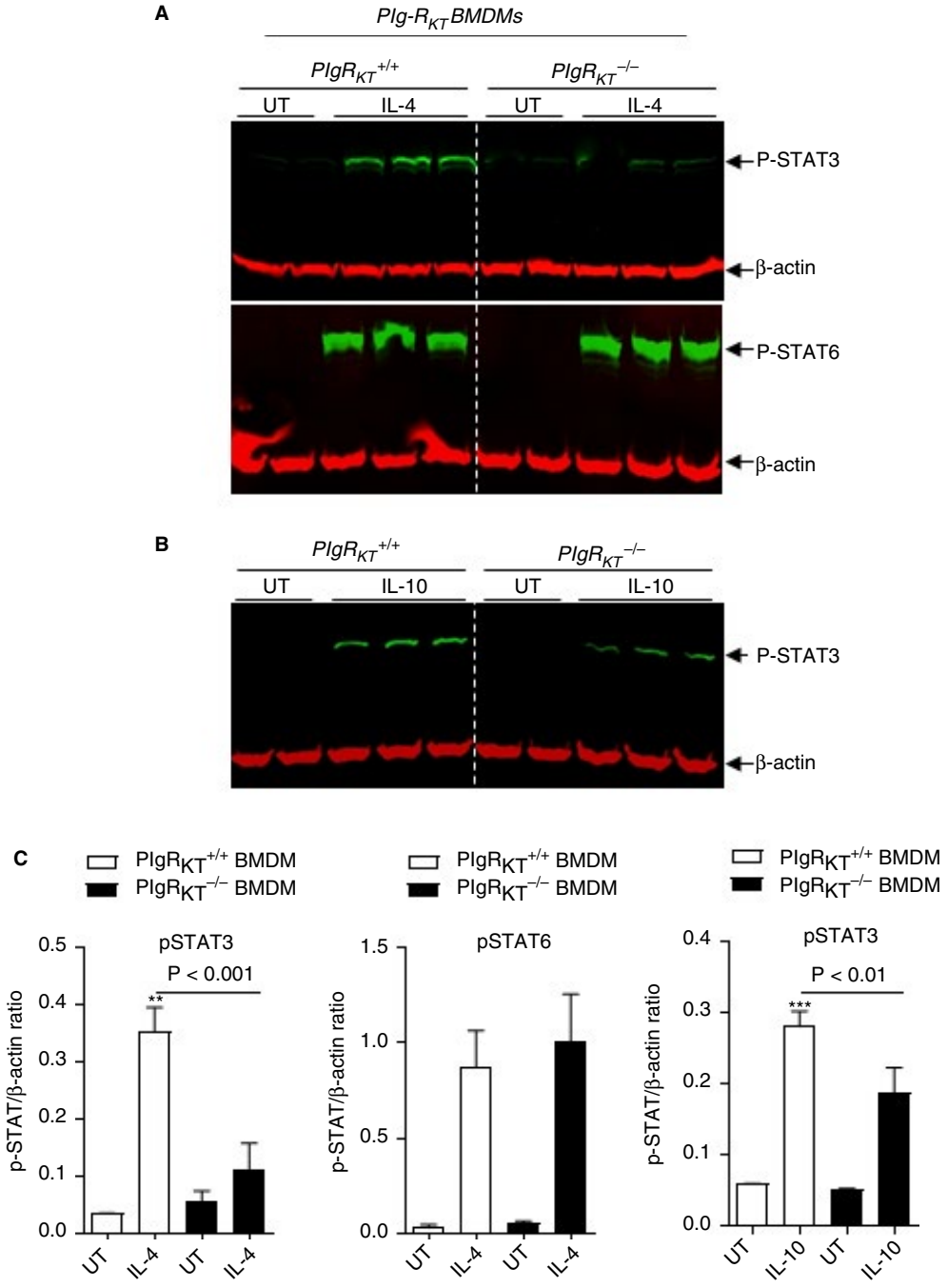

FIGURE 4 Effect of plasmin(ogen) treatment and deletion of Plg-R$_{KT}$ on signal transducer and activator of transcription (STAT) signaling pathways. Bone marrow derived macrophages (BMDMs) from Plg-R$_{KT}^{+/+}$ and Plg-R$_{KT}^{-/-}$ mice were washed three times with serum-free media and then treated with either interleukin (IL)-4 (20 ng/mL), lipopolysaccharide (10 ng/mL) + interferon (10 ng/mL), IL-10 (20 ng/mL), or untreated (UT) for 30 minutes (A, B). Cell lysates were analyzed by western blotting for the indicated antigens. β-actin was used as a loading control. Densitometry analyses are shown (C). This research was originally published in Vago et al[54]

(46%) than female mice.[56] In humans, female sex is associated with higher plasminogen levels.[71] Female murine monocytes express significantly more cell surface Plg-R$_{KT}$ than male monocytes.[39] And the number of monocytes recruited to the peritoneum in the thioglycollate model is markedly greater in female compared with male mice.[39] Thus, females may have a more efficient activation of plasminogen on cell surfaces, which may significantly affect outcomes of many pathological states.

6 | ROLE OF Plg-R$_{KT}$ IN LACTATION

Plg-R$_{KT}$-deficient mice are viable and fertile and offspring of heterozygous matings are born according to the expected Mendelian ratios and there is no effect of genotype on survival.[56] However, Plg-R$_{KT}^{-/-}$ females (on the C57Bl/6J background) exhibit a severe lactation defect, resulting in death of all offspring within 2 days after birth (Figure 5).[43] Pups of Plg-R$_{KT}^{+/-}$ female mice have survival rates

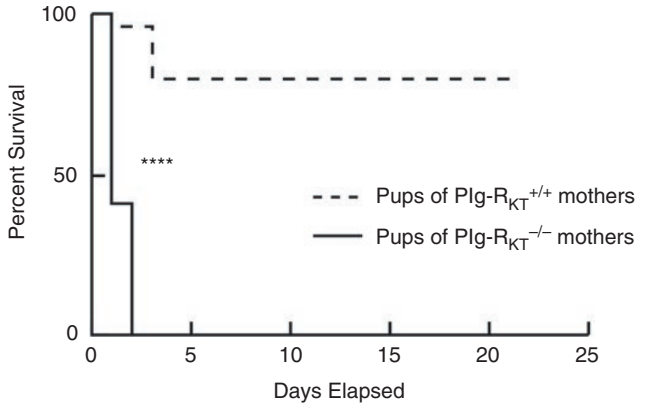

FIGURE 5 Plg-R_{KT} null mice cannot successfully lactate. Survival data are shown for a cohort of offspring of Plg-$R_{KT}^{+/+}$ (dashed lines) and Plg-$R_{KT}^{-/-}$ (solid lines) primiparous female littermates: 48 offspring of Plg-$R_{KT}^{+/+}$ mice and 30 offspring of Plg-$R_{KT}^{-/-}$ mice. **** $P < .0001$ Log-rank (Mantel Cox) test. This research was originally published in Miles et al[43] and is reprinted with permission from John Wiley and Sons

intermediate between offspring of Plg-$R_{KT}^{-/-}$ and Plg-$R_{KT}^{+/+}$ females, indicating a significant maternal gene dosage effect.[56] The effect of maternal genotype on pup survival is not strain-dependent as the lactational defect is also present in Plg-R_{KT}-deficient mice on the 129XS/SVJ background.[43] Pup death is consistent with the inability of Plg-$R_{KT}^{-/-}$ mothers to successfully lactate as milk is not present in the stomachs of offspring of Plg-$R_{KT}^{-/-}$ mothers. In addition, cross fostering demonstrates that the lactation defect is with Plg-$R_{KT}^{-/-}$ mothers.[43] This section reviews evidence for mechanisms by which Plg-R_{KT} regulates lactation.

6.1 | Plg-R_{KT} regulates milk production and ductal histology

Mammary gland masses of Plg-$R_{KT}^{-/-}$ mice are significantly (50%) less than those of Plg-$R_{KT}^{+/+}$ mice. Furthermore, milk production is severely and significantly decreased in mammary glands of Plg-$R_{KT}^{-/-}$ mothers, while there are no genotype-dependent effects on tissue prolactin levels in mammary glands.[43] The alveoli of Plg-$R_{KT}^{-/-}$ glands are strikingly atrophic and disordered and ducts are distended. Amorphous eosinophilic material, suggesting high protein content, is present in alveoli and ducts of Plg-$R_{KT}^{-/-}$ glands.[43]

6.2 | Plg-R_{KT} regulates the composition of the ECM in lactating mammary glands

Lactogenesis requires the appropriate extracellular environment[72-74] and deletion of Plg-R_{KT} results in marked dysregulation of the mammary ECM. Excessive collagen deposition is present within mammary adipose tissue surrounding the alveoli of the Plg-$R_{KT}^{-/-}$ mammary glands and fibrosis is also present within the surrounding adipose tissue of Plg-$R_{KT}^{-/-}$ mammary glands.[43] Consistent with the fibrotic ECM, cellular infiltrates are present within fibrotic areas of Plg-$R_{KT}^{-/-}$ glands, with marked accumulation of macrophages.[43]

Excessive fibrin deposition is present in the fibrotic mammary adipose tissue surrounding the alveoli and is also present within the distended alveoli and ducts of Plg-$R_{KT}^{-/-}$ glands.[43] There are no genotype-dependent effects on glandular levels of plasminogen activators, suggesting that the absence of Plg-R_{KT} results in defective fibrinolysis due to impaired plasminogen activation.

6.3 | Plg-R_{KT} deletion affects fibrosis gene expression signatures in lactating mammary glands

In fibrosis gene transcription profiling of 84 genes in mammary glands harvested 2 days postpartum, 25 genes were identified for which expression in Plg-$R_{KT}^{-/-}$ glands was significantly ($P < .05$) different than Plg-$R_{KT}^{+/+}$ glands by a factor of >two-fold.[43] Upregulation of remodeling enzymes and inhibitors (Mmp2, Mmp9, and Timp2), inflammatory cytokines and chemokines (Il1a, Il4, and TNF) and integrins (α2, β1, β3, β6, and β8) within the mammary tissue was consistent with the marked infiltration of macrophages observed in Plg-$R_{KT}^{-/-}$ glands. In addition, TGFβ3 and its targets, Smad 2 and CTGF (connective tissue growth factor or CCN2) were strikingly upregulated. In addition, Mmp1a was markedly down-regulated while Timp2 was markedly up-regulated in Plg-$R_{KT}^{-/-}$ glands, consistent with extensive collagen deposition in this genotype.

6.4 | Plg-R_{KT} deletion decreases epithelial cell proliferation and increases epithelial cell apoptosis

Epidermal growth factor (EGF) was the most extensively down-regulated gene identified in Plg-$R_{KT}^{-/-}$ glands (12-fold less expression). Of ligands for the EGF receptor (EGFR), EGF is exclusively induced at lactation and is the most abundant of all differentially expressed cytokines and growth factors in luminal epithelial cells of lactating glands.[75] Correspondingly, levels of phospho-EGFR were markedly decreased in Plg-$R_{KT}^{-/-}$ glands (Figure 6). Furthermore, proliferating epithelial cells are abundant in Plg-$R_{KT}^{+/+}$ glands while not detectable in Plg-$R_{KT}^{-/-}$ glands[43] (Figure 7A).

EGF orchestrates increased translation of the pro-survival Bcl-2 family protein, Mcl-1, and genetic deletion of Mcl-1 results in increased epithelial apoptosis.[75] Mcl-1 expression is observed in Plg-$R_{KT}^{+/+}$ glands, but is not detectable in Plg-$R_{KT}^{-/-}$ glands (Figure 7B) and, correspondingly, cleaved (activated) caspase 3 is present in Plg-$R_{KT}^{-/-}$ glands, but not detectable in Plg-$R_{KT}^{+/+}$ glands[43] (Figure 7C).

6.5 | Interplay between plasminogen-dependent and plasminogen-independent functions in lactational development

A role for Plg-R_{KT} in regulating fibrinolysis within mammary glands is supported by the excessive fibrin deposition in Plg-$R_{KT}^{-/-}$ glands.

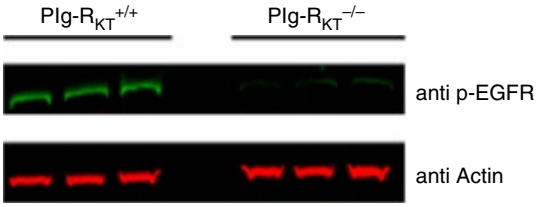

FIGURE 6 Phosphorylated epidermal growth factor receptor (EGFR) content is decreased in Plg-R$_{KT}^{-/-}$ mammary glands. Thoracic mammary glands were harvested from Plg-R$_{KT}^{+/+}$ and Plg-R$_{KT}^{-/-}$ female mice 2 days following parturition. Glands were lysed and electrophoresed on 4% to 12% gradient gels under reducing conditions and western blotted with anti-phosphorylated EGFR. Ratios of pEGFR:Actin were significantly less in Plg-R$_{KT}^{-/-}$ glands (Plg-R$_{KT}^{-/-}$ = 0.34 ± 0.1; Plg-R$_{KT}^{+/+}$ = 0.90 ± 0.01, P = .011, n = 3). There were no genotype effects on EGFR levels. These data have not been published previously

Notably fibrin deposition is also observed in plasminogen-deficient glands at postpartum day 2.[76] The lactation defect in plasminogen-deficient mice is rescued on a fibrinogen heterozygous background.[76] In contrast, fibrinogen heterozygosity does not rescue survival of offspring of Plg-R$_{KT}^{-/-}$ females.[43] Moreover, the severity and histological basis of the lactation defect in Plg-R$_{KT}^{-/-}$ mice is much more pronounced than that of plasminogen-deficient mice.[76,77] Phenotypic differences include: (a) all offspring of Plg-R$_{KT}^{-/-}$ females die by postpartum day 2, whereas 60% of offspring of primiparous plasminogen-deficient females are alive at postpartum day 2 and the rate of total pup death is gradual, with at least 25% pup survival for 17 days;[76] (b) lobuloalveolar development takes place normally in plasminogen-deficient mice and mammary gland histology is indistinguishable from wild type littermates at postpartum day 2; and (c) milk production in plasminogen-deficient mammary glands is normal.[76,77] Thus, although blockade of ducts with fibrin may play a role, it cannot account for the complete failure of lactation and impaired alveologenesis in Plg-R$_{KT}^{-/-}$ mice. It is also noteworthy that, although uPAR associates with Plg-R$_{KT}$,[34,37] we are unaware of reports of a lactation defect in uPAR-deficient mice.

Based on comparison of the lactational phenotype published for plasminogen-deficient mice[76,77] with results with Plg-R$_{KT}^{-/-}$ mice, the influence of Plg-R$_{KT}$ on ECM remodeling can occur by both plasminogen-dependent and plasminogen-independent mechanisms (Figure 8). By promoting plasminogen activation, Plg-R$_{KT}$ can exert anti-fibrotic effects by regulating remodeling of the stromal fibrin scaffold, activation of MMPs for collagen remodeling, and proteolysis of the ECM component entactin/nidogen-1. Plg-R$_{KT}$ may also exert plasminogen-independent anti-fibrotic effects as mammary gland fibrosis is observed at much earlier time points in lactational development of Plg-R$_{KT}^{-/-}$ glands compared with plasminogen-deficient glands.[76] Plg-R$_{KT}$ may also regulate plasminogen-independent ECM remodeling for lobuloalveolar development as lobuloalveolar development proceeds normally in plasminogen-deficient glands.[76,77] In addition, Plg-R$_{KT}$ promotes EGF biosynthesis, in a plasminogen-independent fashion, to allow proliferation of epithelial cells to promote lobuloalveolar development. Furthermore, via promoting EGF biosynthesis, Plg-R$_{KT}$ promotes Mcl-1 translation to inhibit apoptosis and maintain alveolar and ductal structure. Finally, Plg-R$_{KT}$ can promote plasminogen activation on the surfaces of luminal epithelial cells for fibrin surveillance within the alveoli and ducts to maintain alveolar patency. Plg-R$_{KT}$ is, thus, a critical protein required for survival of the species, based on the total failure of lactation in Plg-R$_{KT}^{-/-}$mice. We speculate that this may have major implications for the function of other organs that undergo post-embryonic morphogenesis including kidney,[78] prostate,[79] lacrimal gland,[80] and salivary gland[81] in that Plg-R$_{KT}$ may regulate morphogenesis, particularly epitheliogenesis, in response to pathological challenges.

7 | EMERGING ROLES OF Plg-R$_{KT}$

Plg-R$_{KT}$ is likely to play key roles in the many physiological and pathological processes summarized in section 2.2. Here we highlight emerging roles of Plg-R$_{KT}$ for which published information is available.

7.1 | Lipoprotein(a) catabolism

Lipoprotein(a) (Lp[a]) is an low-density lipoprotein (LDL)-like particle in which apolipoprotein B-100 is covalently bound to an unique lipoprotein, apolipoprotein(a) (apo[a]). Lp(a) is both a risk factor for and a factor causing cardiovascular disease and calcific aortic valve stenosis.[82] The sequence of apo(a) is remarkably homologous to plasminogen[83,84] and Lp(a) competes with plasminogen for the interaction with cell surfaces.[85,86] Recent in vitro studies in which Plg-R$_{KT}$ was either knocked down or overexpressed in liver cells have indicated that Plg-R$_{KT}$ is responsible for the majority of Lp(a) uptake in these cells. Interestingly, after Lp(a) is internalized, the apo(a) moiety then trafficks to recycling endosomes with subsequent apo(a) resecretion, while the LDL particle trafficks to lysosomes where it is degraded[87,88] (see commentary in Yeang et al[89]). Future studies with Plg-R$_{KT}^{-/-}$ mice should aid in elucidating the role of Plg-R$_{KT}$ in Lp(a) metabolism in vivo.

7.2 | Neuroendocrine roles of Plg-R$_{KT}$

7.2.1 | Neurotransmitter release

Catecholaminergic cells, including chromaffin cells of the adrenal medulla and other neurosecretory cells, bind both plasminogen and t-PA, resulting in promotion of plasminogen activation on these cell surfaces.[90] Furthermore, both t-PA and plasminogen are secreted by catecholaminergic cells in response to stimulation with specific secretagogues,[91,92] providing a local neurosecretory environment in which plasmin activity is produced (reviewed in Bai et al[93]). The locally generated plasmin functions in extracellular processing of secreted hormones.[90] As an example of extracellular plasmic processing,

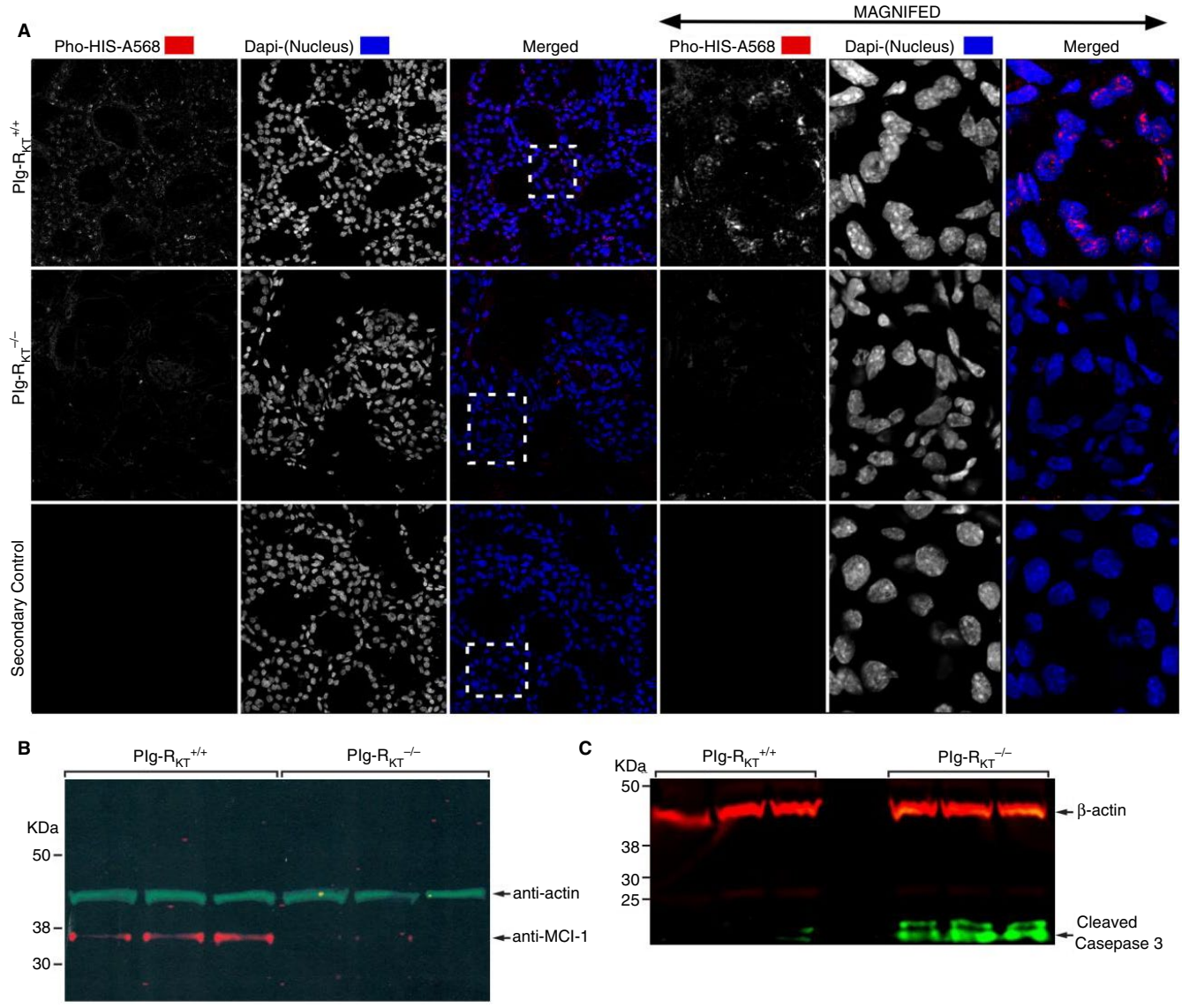

FIGURE 7 Plg-R_{KT} deletion decreases epithelial cell proliferation and increases epithelial cell apoptosis. Mammary glands were harvested 2 days postpartum from Plg-$R_{KT}^{+/+}$ and Plg-$R_{KT}^{-/-}$ female mice. Frozen sections of abdominal mammary glands were immunostained with antibodies against Ser_{28}-phosphorylated histone H3 (Pho-HIS-A568) and Dapi (shown in white for greater clarity). Boxes defined by dashed lines are the areas enlarged in right panels. Confocal images were captured using a Zeiss 710 Laser Scanning Confocal Microscope running the Zen 2016 software. Eight-bit optical image section slices (sampled at 0.3 μm intervals) were collected as 1024 × 1024 images, converted to maximum intensity projections for 2D analysis then imported into: Image Pro Premier, Image J, and LSM Examiner software for further processing and quantitative analysis. A, Thoracic glands were lysed and electrophoresed on 4% to 12% gradient gels under reducing conditions and western blotted with anti-Mcl-1 and anti-βactin (B) or anti-active (cleaved) caspase 3 and anti-βactin (C). This research was originally published in Miles et al[43] and is reprinted with permission from John Wiley and Sons

neurotransmitter release from catecholaminergic cells is negatively regulated by cleavage products that are produced by plasmic proteolysis of the major secretory vesicle core protein, Chromogranin A (CgA).[90,94]

Plg-R_{KT} is prominently expressed in catecholaminergic cells including adrenal medullary chromaffin cells within both murine and human adrenal tissue, as well as in bovine adrenal chromaffin cells, human pheochromocytoma, rat PC12 pheochromocytoma cells, and in murine hippocampus.[37] Overexpression of Plg-R_{KT} results in enhanced plasminogen activation on PC12 cells and, importantly, release of the neurotransmitter norepinephrine is markedly suppressed.[37] Thus, Plg-R_{KT} promotes plasminogen activation to release peptides that feed back to regulate release of catecholamines by these cells and this has major implications for regulation of sympathoadrenal activation and stress responses.[93] Because Plg-R_{KT} is broadly expressed in neuroendocrine and neuronal tissues, these studies additionally suggest a broader paradigm for regulation of local proteolysis and, consequently, neurotransmitter release at many neuroendocrine and neuronal sites.

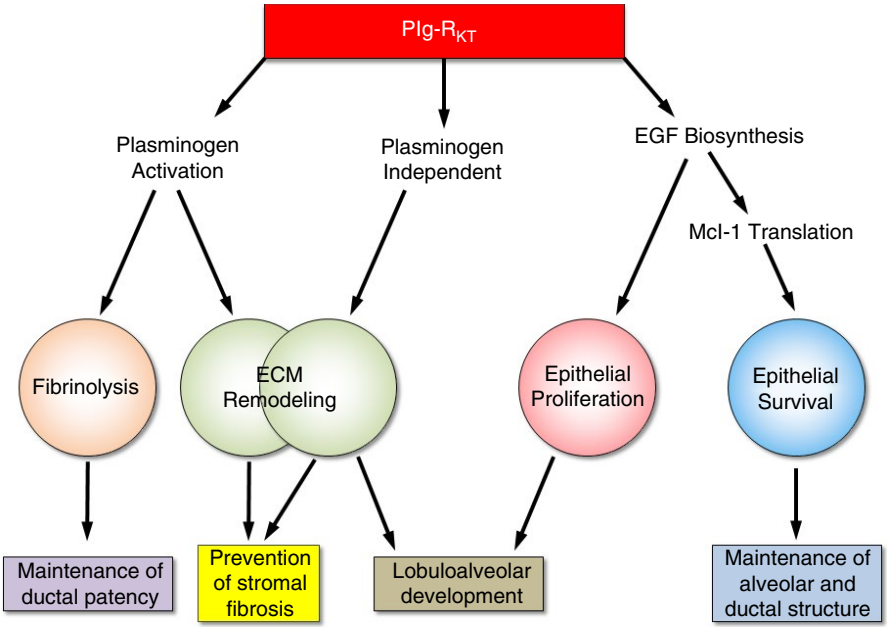

FIGURE 8 Working model for regulation of lactogenesis by Plg-R$_{KT}$. Plg-R$_{KT}$ regulates key steps essential for nomal lactogenesis. By promoting plasminogen activation, Plg-R$_{KT}$ can exert anti-fibrotic effects by regulating remodeling of the stromal fibrin scaffold, activation of matrix metalloproteinases for collagen remodeling, and proteolysis of the extracellular matrix (ECM) component entactin/nidogen-1. Plg-R$_{KT}$ may also exert plasminogen-independent anti-fibrotic effects as mammary gland fibrosis is observed at much earlier time points in lactational development of Plg-R$_{KT}^{-/-}$ glands compared with Plg$^{-/-}$ glands. Plg-R$_{KT}$ may also regulate plasminogen-independent ECM remodeling for lobuloalveolar development as lobuloalveolar development proceeds normally in Plg$^{-/-}$ glands. In addition, Plg-R$_{KT}$ promotes epidermal growth factor (EGF) biosynthesis, in a plasminogen-independent fashion, to allow proliferation of epithelial cells to promote lobuloalveolar development. Furthermore, via promoting EGF biosynthesis, Plg-R$_{KT}$ promotes Mcl-1 translation to inhibit apoptosis and maintain alveolar and ductal structure. (Epithelial proliferation and apoptosis are markedly affected by Plg-R$_{KT}$ deletion but are not affected in Plg$^{-/-}$ glands at day 2 of lactation.) Finally, Plg-R$_{KT}$ can promote plasminogen activation on the surfaces of luminal epithelial cells for fibrin surveillance within the alveoli and ducts to maintain alveolar patency. This diagram has not been published previously

7.2.2 | The inflammatory response in Alzheimer's disease

Inflammation plays a key role in Alzheimer's disease (AD). The role of microglial activation and macrophage activation in the progression of AD is being increasingly recognized.[95] The neuroimmune response exhibits neuroprotective effects by phagocytosis and clearance of amyloid beta (Aβ) from the brain. However, activated microglia and macrophages produce neurotoxic cytokines, chemokines, and reactive oxygen species that lead to neuronal death in the central nervous system (CNS).[96,97] Thus, chronic activation of the neuroimmune system overwhelms its neuroprotective effects by eliciting proinflammatory responses.

Recent studies show that conditional depletion of plasminogen in peripheral blood is highly protective from Aβ deposition and the neuroinflammatory response and is accompanied by decreased microgial/macrophage activation in the 5XFAD murine model of AD.[98] Notably the presence of perivascular macrophages in major vessels of the brain is decreased in plasminogen-depleted AD mice. These studies suggest a previously unappreciated role of circulating plasminogen in AD.[98] Furthermore, conditional depletion of plasminogen reduces migration of perivascular macrophages into the CNS in response to systemic injection of LPS.[99] Blood cells are the source of perivascular macrophages in the CNS[100] and perivascular macrophages show high expression of Plg-R$_{KT}$,[99] suggesting a role for Plg-R$_{KT}$ in plasminogen-dependent macrophage migration in neuroinflammation.

8 | CONCLUSIONS

This review has focused on recently published studies that have revealed in vivo functions of Plg-R$_{KT.}$ Physiologic and pathophysiologic functions of this plasminogen receptor are based on its ability to bind plasminogen, promote plasminogen activation, localize plasmin on cell surfaces, and mediate plasmin-dependent cell signaling. In addition, Plg-R$_{KT}$ may exert plasminogen-independent functions as exemplified by its role in lactational development. Given the predominant role of Plg-R$_{KT}$ in the inflammatory response, future studies of its role in pathophysiologic events involving macrophage and lymphocyte function are warranted. In view of the broad tissue expression of Plg-R$_{KT}$, its roles in a broad array of pathologies involving other tissues should be revealed in future studies.

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

LAM, JPV, LPS, and RJP wrote the paper.

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REFERENCES

1. Miles LA, Plow EF. Binding and activation of plasminogen on the platelet surface. *J Biol Chem*. 1985;260:4303-4311.
2. Miles LA, Parmer RJ. Plasminogen receptors: the first quarter century. *Semin Thromb Hemost*. 2013;39:329-337.
3. Miles LA, Lighvani S, Baik N, et al. New insights into the role of Plg-RKT in macrophage recruitment. *Int Rev Cell Mol Biol*. 2014; 309: 259-302.
4. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. *J Biol Chem*. 1982;257:2912-2919.
5. Han J, Baik N, Kim KH, et al. Monoclonal antibodies detect receptor-induced binding sites in Glu-plasminogen. *Blood*. 2011;118:1653-1662.
6. Gong Y, Kim S-O, Felez J, Grella DK, Castellino FJ, Miles LA. Conversion of glu-plasminogen to lys-plasminogen is necessary for optimal stimulation of plasminogen activation on the endothelial cell surface. *J Biol Chem*. 2001;276:19078-19083.
7. Miles LA, Castellino FJ, Gong Y. Critical role for conversion of glu-plasminogen to Lys-plasminogen for optimal stimulation of plasminogen activation on cell surfaces. *Trends Cardiovasc Med*. 2003;13:21-30.
8. Zhang L, Gong Y, Grella DK, Castellino FJ, Miles LA. Endogenous plasmin converts Glu-plasminogen to Lys-plasminogen on the monocytoid cell surface. *J Thromb Haemost*. 2003;1:1264-1270.
9. Félez J, Miles LA, Fábregas P, Jardi M, Plow EF, Lijnen RH. Characterization of cellular binding sites and interactive regions within reactants required for enhancement of plasminogen activation by tPA on the surface of leukocytic cells. *Thromb Haemost*. 1996;76:577-584.
10. Plow EF, Freaney DE, Plescia J, Miles LA. The plasminogen system and cell surfaces: evidence for plasminogen and urokinase receptors on the same cell type. *J Cell Biol*. 1986;103:2411-2420.
11. Hall SW, Humphries JE, Gonias SL. Inhibition of cell surface receptor-bound plasmin by a_2- antiplasmin and a_2-macroglobulin. *J Biol Chem*. 1991;266:12329-12336.
12. Lu BG, Sofian T, Law RH, Coughlin PB, Horvath AJ. Contribution of conserved lysine residues in the alpha2-antiplasmin C terminus to plasmin binding and inhibition. *J Biol Chem*. 2011;286:24544-24552.
13. Li X, Syrovets T, Simmet T. The serine protease plasmin triggers expression of the CC-chemokine ligand 20 in dendritic cells via Akt/NF-kappaB-dependent pathways. *J Biomed Biotechnol*. 2012;2012:186710.
14. Syrovets T, Lunov O, Simmet T. Plasmin as a proinflammatory cell activator. *J Leukoc Biol*. 2012;92:509-519.
15. Miles LA, Levin EG, Plescia J, Collen D, Plow EF. Plasminogen receptors, urokinase receptors and their modulation on human endothelial cells. *Blood*. 1988;72:628-635.
16. Miles LA, Dahlberg CM, Plescia J, Felez J, Kato K, Plow EF. Role of cell-surface lysines in plasminogen binding to cells: Identification of alpha-Enolase as a candidate plasminogen receptor. *Biochemistry*. 1991;30:1682-1691.
17. Redlitz A, Fowler BJ, Plow EF, Miles LA. The role of an enolase-related molecule in plasminogen binding to cells. *Eur J Biochem*. 1995;227:407-415.
18. Hembrough TA, Vasudevan J, Allietta MM, Glass WF, Gonias SL. A cytokeratin 8-like protein with plasminogen-binding activity is present on the external surfaces of hepatocytes, HepG2 cells and breast carcinoma cell lines. *J Cell Sci*. 1995;108(Pt 3):1071-1082.
19. Hembrough TA, Kralovich KR, Li L, Gonias SL. Cytokeratin 8 released by breast carcinoma cells *in vitro* binds plasminogen and tissue-type plasminogen activator and promotes plasminogen activation. *Biochem J*. 1996;317:763-769.
20. Kassam G, Le BH, Choi KS, et al. The p11 subunit of the annexin II tetramer plays a key role in the stimulation of t-PA-dependent plasminogen activation. *Biochemistry*. 1998;37:16958-16966.
21. Hawley SB, Tamura T, Miles LA. Purification, cloning, and characterization of a profibrinolytic plasminogen-binding protein, TIP49a. *J Biol Chem*. 2001;276:179-186.
22. Herren T, Burke TA, Das R, Plow EF. Identification of histone H2B as a regulated plasminogen receptor. *Biochemistry*. 2006;45:9463-9474.
23. Dudani AK, Ganz PR. Endothelial cell surface actin serves as a binding site for plasminogen, tissue plasminogen activator and lipoprotein(a). *Br J Haematol*. 1996;95:168-178.
24. Miles LA, Andronicos NM, Baik N, Parmer RJ. Cell-surface actin binds plasminogen and modulates neurotransmitter release from catecholaminergic cells. *J Neurosci*. 2006;26:13017-13024.
25. Briens A, Bardou I, Lebas H, et al. Astrocytes regulate the balance between plasminogen activation and plasmin clearance via cell-surface actin. *Cell Discov*. 2017;3(1):17001.
26. Fan Z, Larson PJ, Bognacki J, et al. Tissue factor regulates plasminogen binding and activation. *Blood*. 1998;91:1987-1998.
27. Miles LA, Dahlberg CM, Levin EG, Plow EF. Gangliosides interact directly with plasminogen and urokinase and may mediate binding of these components to cells. *Biochemistry*. 1989;280:9337-9343.
28. Miles LA, Ginsberg MH, White JG, Plow EF. Plasminogen interacts with human platelets through two distinct mechanisms. *J Clin Invest*. 1986;77:2001-2009.
29. Pluskota E, Soloviev DA, Bdeir K, Cines DB, Plow EF. Integrin alphaMbeta2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. *J Biol Chem*. 2004;279:18063-18072.
30. Lishko VK, Novokhatny VV, Yakubenko VP, Skomorovska-Prokvolit HV, Ugarova TP. Characterization of plasminogen as an adhesive ligand for integrins {alpha}M{beta}2 (Mac-1) and {alpha}5{beta}1(VLA-5). *Blood*. 2004.
31. Parkkinen J, Raulo E, Merenmies J, et al. Amphoterin, the 30-kDa protein in a family of HMG1-type polypeptides. Enhanced expression in transformed cells, leading edge localization, and interactions with plasminogen activation. *J Biol Chem*. 1993;268:19726-19738.
32. Kanalas JJ, Makker SP. Identification of the rat Heymann nephritis autoantigen (GP330) as a receptor site for plasminogen. *J Biol Chem*. 1991;266:10825-10829.
33. Miles LA, Plow EF, Waisman DM, Parmer RJ. Plasminogen receptors. *J Biomed Biotechnol*. 2012;2012:130735.
34. Andronicos NM, Chen EI, Baik N, et al. Proteomics-based discovery of a novel, structurally unique, and developmentally regulated plasminogen receptor, Plg-RKT, a major regulator of cell surface plasminogen activation. *Blood*. 2010;115:1319-1330.
35. Miles LA, Andronicos NM, Chen EI, et al. Identification of the novel plasminogen receptor, Plg-RKT. In: Man TK, Flores RJ eds. *Proteomics/Book 1: Human Diseases and Protein Functions*. ISBN 978-953-307-832-8 edn: London: Intech; 2012:219-238.
36. Liu M, Zhang S. A kringle-containing protease with plasminogen-like activity in the basal chordate Branchiostoma belcheri. *Biosci Rep*. 2009;29:385-395.
37. Bai H, Baik N, Kiosses WB, Krajewski S, Miles LA, Parmer RJ. The novel plasminogen receptor, plasminogen receptor(KT)

(Plg-R(KT)), regulates catecholamine release. *J Biol Chem*. 2011;286:33125-33133.
38. Miles LA, Lighvani S, Baik N, et al. The plasminogen receptor, Plg-R(KT), and macrophage function. *J Biomed Biotechnol*. 2012;2012:250464.
39. Thaler B, Baik N, Hohensinner PJ, et al. Differential expression of Plg-RKT and its effects on migration of proinflammatory monocyte and macrophage subsets. *Blood*. 2019;134:561-567.
40. Lighvani S, Baik N, Diggs JE, Khaldoyanidi S, Parmer RJ, Miles LA. Regulation of macrophage migration by a novel plasminogen receptor Plg-RKT. *Blood*. 2011;118:5622-5630.
41. Felez J, Chanquia CJ, Fabregas P, Plow EF, Miles LA. Competition between plasminogen and tissue plasminogen activator for cellular binding sites. *Blood*. 1993;82:2433-2441.
42. Miles LA, Krajewski S, Baik N, et al. Plg-PKT protein is broadly distributed in human and murine tissues. Presented at the 22nd International Congress on Fibronolysis and Proteolysis, Marseille, France. 2014: (CP39)
43. Miles LA, Baik N, Bai H, et al. The plasminogen receptor, Plg-RKT, is essential for mammary lobuloalveolar development and lactation. *J Thromb Haemost*. 2018;16:919-932.
44. Sousa LP, Alessandri AL, Pinho V, Teixeira MM. Pharmacological strategies to resolve acute inflammation. *Curr Opin Pharmacol*. 2013;13:625-631.
45. Serhan CN, Brain SD, Buckley CD, et al. Resolution of inflammation: state of the art, definitions and terms. *FASEB J*. 2007;21:325-332.
46. Ploplis VA, French EL, Carmeliet P, Collen D, Plow EF. Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. *Blood*. 1998;91:2005-2009.
47. Plow EF, Ploplis VA, Busuttil S, Carmeliet P, Collen D. A role of plasminogen in atherosclerosis and restenosis models in mice. *Thromb Haemost*. 1999;82(Suppl 1):4-7.
48. Busuttil SJ, Ploplis VA, Castellino FJ, Tang L, Eaton JW, Plow EF. A central role for plasminogen in the inflammatory response to biomaterials. *J Thromb Haemost*. 2004;2:1798-1805.
49. Gong Y, Hart E, Shchurin A, Hoover-Plow J. Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice. *J Clin Invest*. 2008;118:3012-3024.
50. Swaisgood CM, Schmitt D, Eaton D, Plow EF. In vivo regulation of plasminogen function by plasma carboxypeptidase B. *J Clin Invest*. 2002;110:1275-1282.
51. Ploplis VA, Carmeliet P, Vazirzadeh S, et al. Effects of disruption of the plasminogen gene on thrombosis, growth, and health in mice. *Circulation*. 1995;92(9):2585–2593.
52. Carmo AA, Costa BR, Vago JP, et al. Plasmin induces in vivo monocyte recruitment through protease-activated receptor-1-, MEK/ERK-, and CCR2-mediated signaling. *J Immunol*. 2014;193:3654-3663.
53. Sugimoto MA, Ribeiro AL, Costa BR, et al. Plasmin and Plasminogen induce macrophage reprogramming and regulate key steps of inflammation resolution via Annexin A1. *Blood*. 2017;129(21):2896-2907.
54. Vago JP, Sugimoto MA, Lima KM, et al. Plasminogen and the plasminogen receptor, Plg-RKT, regulate macrophage phenotypic, and functional changes. *Front Immunol*. 2019;10:1458.
55. Herren T, Burke TA, Jardi M, Felez J, Plow EF. Regulation of plasminogen binding to neutrophils. *Blood*. 2001;97:1070-1078.
56. Miles LA, Baik N, Lighvani S, et al. Deficiency of plasminogen receptor, Plg-RKT, causes defects in plasminogen binding and inflammatory macrophage recruitment in vivo. *J Thromb Haemost*. 2017;15:155-162.
57. Sousa LP, Lopes F, Silva DM, et al. PDE4 inhibition drives resolution of neutrophilic inflammation by inducing apoptosis in a PKA-PI3K/Akt-dependent and NF-kappaB-independent manner. *J Leukoc Biol*. 2010;87:895-904.
58. Vago JP, Nogueira CR, Tavares LP, et al. Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. *J Leukoc Biol*. 2012;92:249-258.
59. Syrovets T, Jendrach M, Rohwedder A, Schule A, Simmet T. Plasmin-induced expression of cytokines and tissue factor in human monocytes involves AP-1 and IKKbeta-mediated NF-kappaB activation. *Blood*. 2001;97:3941-3950.
60. Li Q, Laumonnier Y, Syrovets T, Simmet T. Plasmin triggers cytokine induction in human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol*. 2007;27:1383-1389.
61. Zalfa C, Azmoon P, Mantuano E, Gonias SL. Tissue-type plasminogen activator neutralizes LPS but not protease-activated receptor-mediated inflammatory responses to plasmin. *J Leukoc Biol*. 2019;105:729-740.
62. Akdis M, Aab A, Altunbulakli C, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. *J Allergy Clin Immunol*. 2016;138:984-1010.
63. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol*. 2010;10:170-181.
64. Vago JP, Tavares LP, Garcia CC, et al. The role and effects of glucocorticoid-induced leucine zipper in the context of inflammation resolution. *J Immunol*. 2015;194:4940-4950.
65. Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41(1):14-20.
66. Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol*. 2014;14:166-180.
67. Rosenwald M, Koppe U, Keppeler H, et al. Serum-derived plasminogen is activated by apoptotic cells and promotes their phagocytic clearance. *J Immunol*. 2012;189:5722-5728.
68. Das R, Ganapathy S, Settle M, Plow EF. Plasminogen promotes macrophage phagocytosis in mice. *Blood*. 2014;124(5):679-688.
69. Miles LA, Parmer RJ. Setting the table for macrophages. *Blood*. 2014;124(5):665-666.
70. Borg RJ, Samson AL, Au AE, et al. Dendritic cell-mediated phagocytosis but not immune activation is enhanced by plasmin. *PLoS One*. 2015;10:e0131216.
71. Ma Q, Ozel AB, Ramdas S, et al. Genetic variants in PLG, LPA, and SIGLEC 14 as well as smoking contribute to plasma plasminogen levels. *Blood*. 2014;124(20):3155-3164.
72. Streuli CH, Bailey N, Bissell MJ. Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. *J Cell Biol*. 1991;115:1383-1395.
73. Boudreau N, Sympson CJ, Werb Z, Bissell MJ. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science*. 1995;267:891-893.
74. Pullan S, Wilson J, Metcalfe A, et al. Requirement of basement membrane for the suppression of programmed cell death in mammary epithelium. *J Cell Sci*. 1996;109(Pt 3):631-642.
75. Fu NY, Rios AC, Pal B, et al. EGF-mediated induction of Mcl-1 at the switch to lactation is essential for alveolar cell survival. *Nat Cell Biol*. 2015;17:365-375.
76. Green KA, Nielsen BS, Castellino FJ, Romer J, Lund LR. Lack of plasminogen leads to milk stasis and premature mammary gland involution during lactation. *Dev Biol*. 2006;299:164-175.
77. Lund LR, Bjorn SF, Sternlicht MD, et al. Lactational competence and involution of the mouse mammary gland require plasminogen. *Development*. 2000;127:4481-4492.
78. Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK. Branching morphogenesis and kidney disease. *Development*. 2004;131:1449-1462.
79. Thomson AA, Marker PC. Branching morphogenesis in the prostate gland and seminal vesicles. *Differentiation*. 2006;74:382-392.
80. Gromova A, Voronov DA, Yoshida M, et al. Lacrimal gland repair using progenitor cells. *Stem Cells Transl Med*. 2017;6:88-98.

81. Patel VN, Rebustini IT, Hoffman MP. Salivary gland branching morphogenesis. *Differentiation*. 2006;74:349-364.
82. Tsimikas S. A test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies. *J Am Coll Cardiol*. 2017;69:692-711.
83. Eaton DL, Fless GM, Kohr WJ, et al. Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. *Proc Natl Acad Sci USA*. 1987;84:3224-3228.
84. McLean JW, Tomlinson JE, Kuang WJ, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature*. 1987;330:132-137.
85. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF. A potential basis for the thrombotic risks associated with lipoprotein(a). *Nature*. 1989;339:301-303.
86. Hajjar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. *Nature*. 1989;339:303-305.
87. Sharma M, Redpath GM, Williams MJ, McCormick SP. Recycling of apolipoprotein(a) After PlgRKT-mediated endocytosis of lipoprotein(a). *Circ Res*. 2017;120:1091-1102.
88. McCormick SPA, Schneider WJ. Lipoprotein(a) catabolism: a case of multiple receptors. *Pathology*. 2019;51:155-164.
89. Yeang C, Gordts PL, Tsimikas S. Novel Lipoprotein(a) catabolism pathway via apolipoprotein(a) recycling: adding the plasminogen receptor PlgRKT to the list. *Circ Res*. 2017;120:1050-1052.
90. Parmer RJ, Mahata M, Gong Y, et al. Processing of chromogranin A by plasmin provides a novel mechanism for regulating catecholamine secretion. *J Clin Invest*. 2000;106:907-915.
91. Parmer RJ, Mahata M, Mahata S, Sebald MT, O'Connor DT, Miles LA. Tissue plasminogen activator (t-PA) is targeted to the regulated secretory pathway: catecholamine storage vesicles as a reservoir for the rapid release of t-PA. *J Biol Chem*. 1997;272:1976-1982.
92. Gualandris A, Jones TE, Strickland S, Tsirka SE. Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. *J Neurol Sci*. 1996;16:2220-2225.
93. Bai H, Nangia S, Parmer RJ. The plasminogen activation system and the regulation of catecholaminergic function. *J Biomed Biotechnol*. 2012;2012:721657.
94. Jiang Q, Taupenot L, Mahata SK, et al. Proteolytic cleavage of chromogranin A (CgA) by plasmin. *J Biol Chem*. 2001;276(27):25022-25029.
95. Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. *Nat Immunol*. 2015;16:229-236.
96. Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators Inflamm*. 2013;2013:480739.
97. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J Cell Biol*. 2018;217:459-472.
98. Baker SK, Chen ZL, Norris EH, Revenko AS, MacLeod AR, Strickland S. Blood-derived plasminogen drives brain inflammation and plaque deposition in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA*. 2018;115:E9687-E9696.
99. Baker SK, Chen ZL, Norris EH, Strickland S. Plasminogen mediates communication between the peripheral and central immune systems during systemic immune challenge with lipopolysaccharide. *J Neuroinflammation*. 2019;16:172.
100. Soulas C, Donahue RE, Dunbar CE, Persons DA, Alvarez X, Williams KC. Genetically modified CD34+ hematopoietic stem cells contribute to turnover of brain perivascular macrophages in long-term repopulated primates. *Am J Pathol*. 2009;174:1808-1817.

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