

# New Insight on the Role of Plasminogen Receptor in Cancer Progression

Seema Kumari and Ramarao Malla

Cancer Biology Lab, Department of Biochemistry, Institute of Science, GITAM University, Visakhapatnam, Andhra Pradesh, India.

## ABSTRACT

**OBJECTIVE:** Plasminogen system plays a crucial role in physiological and pathological events related to tissue regeneration, wound healing, immune response, angiogenesis, invasion and metastasis. It gets activated when plasminogen associates with its cell surface receptors. Latest information on some of the well-explored plasminogen receptors such as annexin II-S100A10, cytokeratin 8,  $\alpha$ -enolase, plasminogen receptor (KT) (Plg-R(KT)) and histone H2B has been discussed in the present review. These receptors can pave the way for effective new therapeutic and diagnostic strategies to counteract malignant diseases.

**CONCLUSION:** The present review concludes the key role of plasminogen receptors in extracellular matrix degradation, infiltration into surrounding tissues, neovascularization, invasion, metastasis and drug resistance. This review also discusses the possible effect of blocking these plasminogen receptors with monoclonal antibodies and DNA-based vaccination or silencing plasminogen receptor gene using small interfering RNA or short hairpin RNA to counteract cancer invasion and metastasis.

**KEYWORDS:** plasminogen receptor, Plg-RKT, annexin II-S100A10, cancer progression, metastasis

**CITATION:** Kumari and Malla. New Insight on the Role of Plasminogen Receptor in Cancer Progression. *Cancer Growth and Metastasis* 2015;8 35–42  
doi:10.4137/CGM.S27335.

**TYPE:** Review

**RECEIVED:** April 9, 2015. **RESUBMITTED:** June 24, 2015. **ACCEPTED FOR PUBLICATION:** June 29, 2015.

**ACADEMIC EDITOR:** Marc D. Basson, Editor in Chief

**PEER REVIEW:** Nine peer reviewers contributed to the peer review report. Reviewers' reports totaled 1,585 words, excluding any confidential comments to the academic editor.

**FUNDING:** Authors disclose no funding sources.

**COMPETING INTERESTS:** Authors disclose no potential conflicts of interest.

**COPYRIGHT:** © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

**CORRESPONDENCE:** rrmalla@gitam.edu

Paper subject to independent expert blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

## Introduction

Plasminogen receptors are widely distributed and form a diverse group of cell surface proteins. These receptors are commonly expressed on cell surfaces, where they can interact with plasminogen and plasmin. Plasminogen is a 90-kDa glycoprotein secreted from the liver. It is a key zymogen protease which regulates extracellular proteolytic events such as fibrinolysis and activation of growth factor. It has N-terminal glutamic acid (Glu) residue and five Kringle regions containing lysine-binding sites (LBS). The LBS arbitrate their binding to cell surface receptor and activation along with a C-terminal protease domain. Plasminogen is activated by various tissue-degrading proteases, including high-affinity activators such as tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), causing activation of plasmin. Plasmin is a serine protease involved in the degradation of the extracellular matrix (ECM) by activating matrix metalloproteinases (MMPs).<sup>1</sup> Cell-associated plasmin proteolysis contributes both to physiological and pathophysiological processes such as tissue remodeling and embryogenesis, invasion, metastasis and inflammation.<sup>2,3</sup> An experiment conducted by Lund et al reported that knock-out mice for uPA and tPA were less impaired in wound healing when compared to plasminogen-deficient mice. This indicates that additional plasminogen activators

such as factor XIa factor XIIa and kallikrein provide a sufficient plasmin to sustain the healing process even in the absence of uPA and tPA.<sup>4</sup>

Plasminogen receptors exhibit differential number of binding sites ranging from  $10^5$  to  $10^7$  per cell, as well as divergence in expression on cell types such as  $\alpha$ M $\beta$ 2-integrin expression is confined to leukocytes and annexin A2/p11 to endothelial cells (ECs). The binding affinity of plasminogen on U937, a human lymphoma cell, was  $6.87 \times 10^4$  at 37°C and decreased to 1/4 at 4°C due to a decrease in plasminogen receptor at this temperature, whereas in GM1380, a fibroblast cell, there was not much difference in plasminogen-binding affinity with a change in temperature.<sup>5</sup> However, histone H2B is abundant on the surface of human umbilical vein endothelial cells. This broad binding ability and expression allows plasminogen receptors to perform diverse biological functions, including fibrinolysis, inflammation, wound healing and angiogenesis.<sup>6</sup> Plasminogen receptors include integrins, urokinase-type plasminogen activator receptors (uPAR) and annexin II, which localizes to the leading edge of migrating cells and creates an advantageous microenvironment, in which one plasminogen receptor is particularly capable of plasminogen activation. However, a recent report has demonstrated



that cell surface-bound plasminogen receptor can be efficiently activated by uPA bound to another cell.<sup>7</sup> In addition, new plasminogen receptors can be elicited by plasmin itself, which depends on the availability of uPA on the cell surface. As a consequence, a cell type may express a variety of plasminogen receptors, but only few can be differentially upregulated and exploited to mediate a specific cellular response. Cellular recruitment is a complex process requiring activation of different intracellular signaling pathways. Diverse plasminogen receptors activate distinct signaling events and cause efficient cell migration. Therefore, blocking of the signaling response induced by any one plasminogen receptor may lead to suppressed signaling and diminished cell migration.

The present review focuses on the importance of plasminogen receptors in activation of the plasminogen system, which plays a key role in cancer progression and metastasis. Thus, blocking plasminogen receptors may be a promising strategy to counteract invasion and metastasis.

### Activation of Plasminogen System by Receptors

The plasminogen system plays a key role in tissue regeneration, wound healing, tissue involution, immune response, angiogenesis, cancer invasion and metastasis.<sup>8–11</sup> The plasminogen system is involved in the regulation of plasmin expression, which promotes activation of laminin, perlemin, fibronectin, pro-uPA and pro-matrix metalloproteinases (pro-MMPs). All the plasminogen receptors have LBS, ie, C-terminal lysine or an internal amino acid residue that mimics C-terminal lysine for binding with plasminogen and plasmin. Plasminogen receptors increase plasminogen activation by either uPA or tPA, thus enhancing the catalytic efficiency of plasmin and also protecting bound plasmin from inactivation by plasmin inhibitors such as  $\alpha$ 2-antiplasmin and  $\alpha$ 2-macroglobulin.<sup>12</sup> In fact, some plasminogen receptors are reported to have one or more functional characteristics. Differences in the affinity of a specific subset of plasminogen receptors for plasminogen might be due to the influence of local conformation or amino acid residues adjacent to LBS-binding residue within receptor. The affinities for ligand appears to be similar in the case of many plasminogen receptors that exploit an internal residue to hold plasminogen with the potential exception of annexin A2/p11 heterotetramer, where the proximity of multiple plasminogen-binding sites within a single molecular species could substantially enhance affinity.<sup>13</sup>

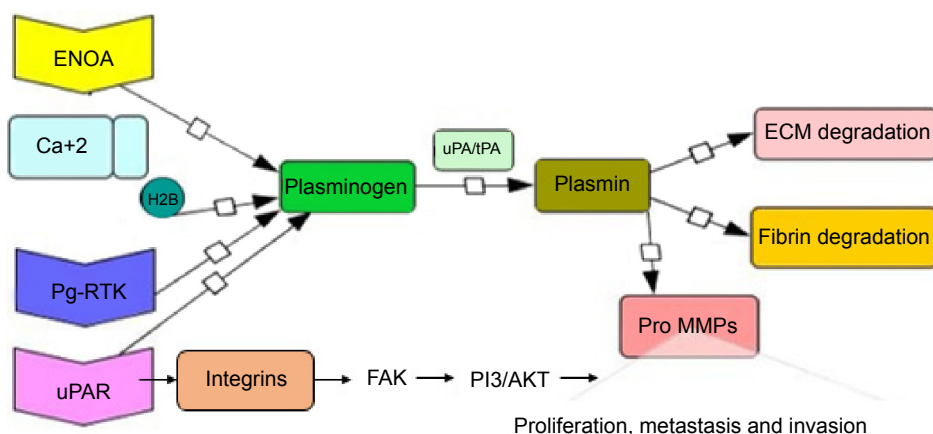
### Plasminogen System and Metastasis

The plasminogen system promotes tumor metastasis by several different mechanisms. One of the well-explored mechanisms is the uPA and uPAR system, which initiates the activation of MMPs as well as the conversion of plasminogen to plasmin followed by ECM degradation and reduced cellular interaction. Expression of uPA and uPAR is regulated by

multiple factors such as mitogen, growth factors, oncogenes v-Src and v-Ras, cytokines, protein kinase C and also on ligation of integrin with extracellular matrix protein. Binding of uPA to uPAR can activate Ras-Raf-MEK-ERK pathway. The FAK has been implicated to mediate signal transduction events initiated by integrins through a recruitment of c-Src or other Src family tyrosine kinases. uPA-induced Ras-ERK signaling pathway is dependent on the downstream effectors Raf and MEK. uPA initiates cell migration via Rho-Rho kinase pathway, which helps to promote Ras-ERK-stimulated cell migration.<sup>14</sup>

### Plasminogen Receptors in Cancer Progression

Plasminogen receptors play a role in the proliferation, migration and metastasis of tumor cells in many cancer types and may serve as prognostic and diagnostic markers.<sup>15</sup> They are involved in mediating colocalization of plasminogen and its activators such as uPA and tPA on cell surfaces and markedly decrease the Km for plasminogen activation. Plasminogen receptors are expressed on the cell surface of most tumors and their expression frequently correlates with cancer diagnosis, survival and prognosis. Notably, they can trigger multiple specific immune responses in cancer patients, highlighting their role as tumor-associated antigens.<sup>16</sup> Except red blood cells (RBCs), plasminogen receptors are broadly distributed on the cell surface with a high binding capacity ( $3 \times 10^7$  molecules per cell).<sup>17,18</sup> Cell surface receptors loaded with plasmin, which is protected from inhibitors, play a key role in cancer progression.<sup>18,19</sup> Plow et al reported that plasmin enhances phagocytosis mediated by macrophages.<sup>20</sup> Therefore, macrophage-mediated phagocytosis of cancerous cells is an important strategy in cancer therapy.<sup>21</sup> In another study on MDA-MB-231 cells, it was analyzed that lipid raft (LR) region of cell membranes showed the predominance of ANG (angiogenin), annexin II and S100A10 were as nonlipid raft (NLR) regions was predominant with uPAR, suggesting that ANG interacts with uPAR at the junctions of LR and NLR regions. uPAR interacts with uPA causing plasmin formation and FAK phosphorylation, which are necessary for tumor migration and invasion.<sup>22</sup> Some well-explored and well-established plasminogen receptors in cancer metastasis and invasion are shown in Figure 1.<sup>23–27</sup> uPAR is a 50- to 60-kDa extracellular glycoprotein, rich in cysteine and congregated in lipid rafts. uPAR is composed of three homologous domains D1, D2, and D3 belonging to the Ly-6/uPAR/alpha-neurotoxin protein domain family, which is attached to the cell membrane by a glycosyl phosphatidyl inositol (GPI) anchor and is responsible for high intramembrane mobility. uPAR binds with high affinity to uPA, pro-uPA and the ECM protein vitronectin (VN). The crystal structure of a soluble form of human uPAR reveals that the receptor-binding module of uPA engages the uPAR central cavity, thus leaving the external receptor



**Figure 1.** Plasminogen receptors and metastasis. Schematic representation of the plasminogen receptors involved in activation of plasmin in the presence of plasminogen and uPA. uPAR along with integrins activates FAK signaling, which activates PI3/AKT and on other side plasmin promotes ECM degradation, fibrin degradation, and activation of pro-MMPs, which finally leads to pathophysiological processes, such as cell proliferation, metastasis and invasion.

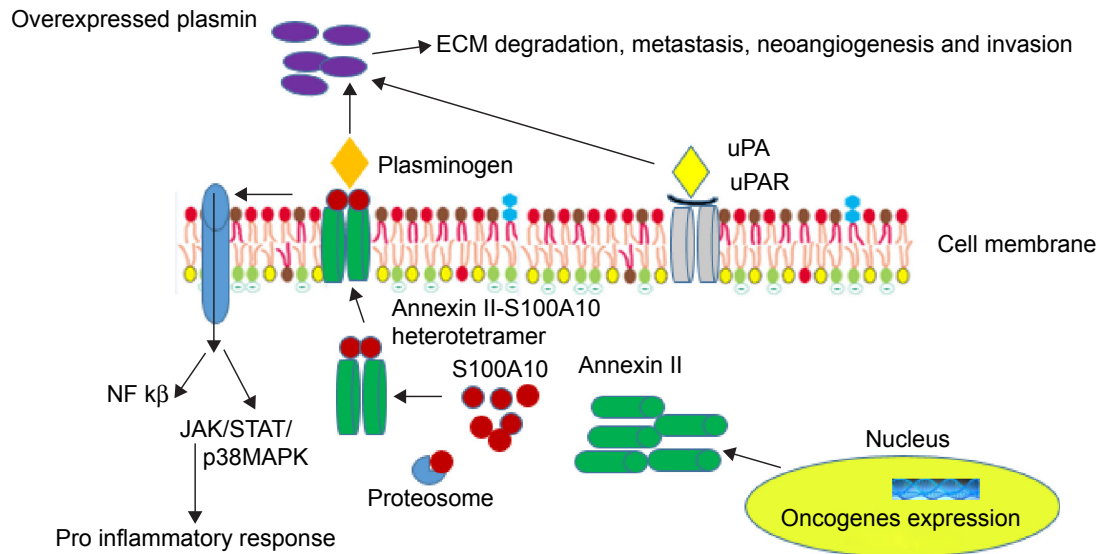
surface accessible for VN and integrins. Somatic mutation of the X chromosome *PIG-A* gene in hematopoietic stem cells cannot synthesize the GPI moiety, which anchors many different types of proteins to the cell membrane. uPAR, which is a GPI-anchored protein expressed on activated monocytes and granulocytes, plays an important role in fibrinolysis by promoting the conversion of urokinase to its active form. Increased levels of soluble uPAR lacking the GPI anchor is associated with paroxysmal nocturnal hemoglobinuria (PNH) as well as in patients with certain types of malignancy. The absence of membrane uPAR on monocytes, granulocytes and platelets might contribute to thrombosis in PNH.<sup>14</sup>

### Annexin AII–S100A10 Heterotetrameric Complex

Annexin II (p36) is a pleiotropic, calcium-regulated, phospholipid-binding protein located in the cytoplasm, plasma membrane and the nucleus. It is grouped under annexin family with a molecular weight (MW) of 36 kDa. It is characterized by the canonical annexin folds, which consists of the amino acid sequence G-X-G-T-(38)-(D/E).<sup>28</sup> It exists as a soluble monomer or stable heterotetramer form without carboxyl-terminal lysine. It acts as a plasminogen receptor by binding with S100A10 (p11), a member of the S100 family protein. This stable heterotetrameric complex possesses two copies of a 36-kDa-heavy chain (p36) of annexin II and two copies of 11-kDa-light chain (p11) of S100A10. The C-terminal lysine residue of S100A10 acid protein forms a binding site for plasminogen activators, plasminogen and plasmin. The role of annexin II and S100A10 is interdependent, where annexin II anchor S100A10 on the cell surface and form ubiquitin-mediated degradation and in turn S100A10 imparts plasminogen-binding activity.

Majority of intracellular S100A10 is found in association with annexin II and the excess of S100A10 undergoes

proteasomal degradation as shown in Figure 2. In a heterotetrameric complex, S100A10 dimer is positioned in the center with annexin II flanking on both sides. After phosphorylation at Tyr-23 through a Src-like tyrosine kinase-dependent mechanism with conformational changes, this heterotetrameric form of annexin II is translocated to the cell surface. However, expression of annexin II on the cell surface is modulated by a different mechanism.<sup>29</sup> At the endothelial cell surface, heterotetramer form AII–S100A10 (p11) accelerates tPA-dependent activation of the fibrinolytic protease plasmin. Previous report suggests that annexin II–S100A10 heterotetramer plays a crucial role in pathological conditions such as inflammation, thrombosis, autoimmune disease, and cancer progression.<sup>30</sup> It has also been reported that cancer cells overexpress annexin II–S100A10 complex, which causes an increase in the formation of plasmin, thus enhancing ECM degradation, infiltration into surrounding tissues, neovascularization, invasion, metastasis and drug resistance.<sup>31–34</sup> Annexin II–S100A10 heterotetramer contributes to cancer invasion and metastasis by acting as a coreceptor for plasminogen, tPA, and pro-cathepsin B.<sup>34</sup> Studies conducted by Connell et al<sup>35</sup> and Swisher et al<sup>36</sup> suggest that in S100A10-deficient mice, macrophage invasion across an inflammatory peritoneal membrane was decreased with a decrease in the generation of plasmin and MMP-9. This indicates that annexin II–S100A10 heterotetramer dramatically affects tumor cell-mediated pericellular proteolysis, tumor invasiveness and metastasis. It has been reported that overexpression of cell surface annexin II is associated with an increase in both mRNA and total cellular expression, suggesting translational regulation.<sup>37</sup> Zhang et al<sup>38</sup> reported that small interfering RNA (siRNA) targeting annexin II not only decreased annexin II messenger RNA and protein levels but also downregulated the levels of S100A10 and c-Myc in invasive breast cancer. The silencing of annexin II inhibited tPA-dependent plasmin generation and reduced cell motility.



**Figure 2.** Schematic representation of overexpressed annexin II–S100A10 heterotetramer in cancer cells. Plasminogens bind to the S100A10 domain of heterotetramer and uPA binds to uPAR and causes overexpression of plasmin, leading to ECM degradation, invasion, and metastasis.

Anti-annexin II antibodies reduce cancer progression by affecting metastasis. Annexin II-specific targets have promising therapeutic benefits in animal models, but are yet to be elucidated in human trials.<sup>39</sup>

### Cytokeratin 8

Cytokeratin 8 (CK8) is a 52-kDa basic and one of the major structural proteins forming cytoplasmic network of intermediate filaments with variable expression on epithelial cells, differentiation stage and epithelial cell-derived neoplasms.<sup>40</sup> Cytokeratin 18 (CK18) is a 45-kDa acidic intermediate filament protein that codes for a type I intermediate filament protein. It is normally coexpressed with CK8 and is found in most simple ductal and glandular epithelia. Constantly, coexpression of CK8 and CK18 is important for the formation of intermediate keratin filament on epithelial and cancer cells.<sup>41</sup> CK8/18 are associated with flexible intracellular scaffolding in order to stabilize the structure of cytoplasm and mitochondria. It is also required to maintain tissue integrity and is involved in apoptosis and cell cycle progression. CK8/18 are typically coexpressed as the primary cytokeratin pairs in simple epithelial cells and their expression is maintained during malignant transformation.<sup>42</sup> Studies on different cancer cells have proved that expression of CK8/18 varies based on posttranslational modifications, including site-specific phosphorylation, O-GlcN-arylation and acetylation. These modifications regulate the solubility, organization and stability of the filament, emphasizing the role of CK8/18 in cancer progression.<sup>43</sup> CK8 exhibits a unique feature among cytokeratins by having a carboxyl-terminal lysine. Recently, it has been demonstrated that CK8 is a primary plasminogen-binding protein on the

plasma membrane.<sup>44</sup> A most acceptable mechanism for the role of CK8 in cancer progression is activation of plasminogen. In breast cancer cells, it has been reported that it acts as a receptor for uPA, which suggests a model in which CK8 in complex with uPA, plasminogen, and fibronectin constitutes a signaling platform capable of modulating invasion, metastasis, cell adhesion, and growth.<sup>45</sup>

Studies using specific antibodies have revealed that both plasminogen and CK8 are located on the surfaces of certain epithelial cells, including hepatocytes, hepatocellular carcinoma cells and various breast cancer cells. This novel study has demonstrated the mechanism of protein penetration through the plasma membrane or binding of secreted CK8 to other cell surface molecules. *In vitro* and *in vivo* studies have also demonstrated that cancer cells are known to secrete plasminogen-binding CK8-containing protein complexes. These complexes bind with plasminogens as well. The plasminogen-binding activity of CK8 is unique among intermediate filament proteins, probably because its sequence includes a carboxyl-terminal Lys residue. However, a CK8 mutant that lacks the C-terminal Lys still binds with plasminogen, albeit with decreased affinity. CK18 does not bind with plasminogen; however, CK8 and CK18 equally bind to tissue-type plasminogen activators (tPA), which may be important in the mechanism, whereby CK8/18 complex promotes plasminogen activation by tPA. High levels of CK8 expression are associated with an increased migration and invasion of certain cancer cells. These correlations are most easily explained by the function of intermediate filament proteins in determining the rigidity of the cytoskeleton; however, the function of cell surface CK8 as a plasminogen receptor merits consideration.<sup>46</sup>

**Table 1.** Comparison between different plasminogen receptors and plasminogen activators in terms of MW, specificity, and functions.

| COMPONENTS          | MOLECULAR WEIGHT (kDa) | SPECIFICITY   | FUNCTION  |
|---------------------|------------------------|---|---|
| Annexin AII         | 36                     | Characterized by canonical annexin folds and do not contain carboxyl-terminal lysine  | Formation of plasmin, enhancing ECM degradation, infiltration into surrounding tissues, neovascularization, invasion, and metastasis  |
| S100A10             | 11                     | Contain C-terminal lysine and binds with annexin II to form plasminogen receptor  | Cancer invasion and metastasis by acting as a co-receptor for plasminogen, tPA and pro cathepsin B  |
| Cytokeratin 8 (CK8) | 52                     | Major structural proteins in forming cytoplasmic network of intermediate filaments with variable expression on epithelial cell, differentiation stage and epithelial cell derived neoplasms | In cancer progression it activates plasminogen which modulates invasion, metastasis, cell adhesion and growth   |
| Enolase (ENOA)      | 48                     | It is highly conserved cytosolic ubiquitous metalloenzyme   | It is involved in activation of plasmin, ECM degradation and also supports anaerobic proliferation induce hypoxic stress through hypoxia responsive element in various cancer cells   |
| Plg-RKT             | 17.3                   | Only integral membrane plasminogen receptor which contains two transmembrane helices with a C-terminal lysine residue as the integral membrane protein                                      | It is also involved in human monocytes chemotaxis and chemokinesis. Plg-RKT also plays an important role in macrophage migration depends activation of matrix metalloproteases (MMPs)   |
| Histone H2B         | 17                     | It is basic organizational unit of chromosomal DNA. It is a membrane protein highly expressed on cell-surface of blood cells  | In cancer cells it activates lymphocytes during apoptosis and also involved in cancer progression   |
| uPA                 | 31.1                   | It is a serine proteases, primary physiological substrate is plasminogen, which is an inactive form (zymogen) of the serine protease plasmin  | uPA has a multifunctional in the neoplastic evolution, affecting tumor angiogenesis, malignant cell proliferation, adhesion and migration, intravasation and growth at the metastatic site  |
| tPA                 | 70                     | Is a serine protease found on endothelial cells of blood vessels. It catalyzes the conversion of plasminogen to plasmin   | Plasmin is involved in ECM degradation directly because it is a broad substrate proteinase that can degrade most proteins within the ECM like fibronectin, laminin, and proteoglycans. Plasmin also acts on the ECM indirectly by activating MMPs |
| XIa                 | 160                    | It is called as plasma thromboplastin antecedent (PTA), it is an endopeptidase  | It is activated by factor XIIa and involved in blood clotting. Involved in activation of plasminogen to plasmin   |
| XIIa                | 80                     | Hageman Factor, Endopeptidase   | Binds to exposed collagen at site of vessel wall injury, activated by high-MW kininogen and kallikrein. Involved in activation of plasminogen to plasmin  |
| Kallikrein          | 86                     | Kallikreins are serine proteases  | Activation of factor XII activation, necessary in factor XIIa activation of XI, precursor for bradykinin. It also activates uPA which is important in activation of plasminogen   |

### Enolase

Enolase (ENOA; 2-phospho-D-glycerate hydrolase) is a highly conserved cytosolic ubiquitous metalloenzyme with a MW of 48 kDa. It catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate in the glycolytic pathway. It exists in three different isoforms, namely, alpha, beta, and gamma based on three independent loci ENO1, ENO2, and ENO3, respectively. These isoforms are expressed in a tissue-specific manner.  $\alpha$ -Enolase (ENOA) is present in almost all adult tissues,  $\beta$ -enolase is expressed in muscle tissues, and  $\gamma$ -enolase

is found in neurons and neuroendocrine.<sup>47</sup> ENOA exists as a homodimer or heterodimer, consisting of alpha-alpha, alpha-beta, and alpha-gamma subunits. All these forms act as plasminogen receptors.<sup>48,49</sup> ENOA as a plasminogen receptor is predominantly responsible for plasminogen activation via C-terminal lysine. It is involved in the activation of plasmin and in ECM degradation and also supports anaerobic proliferation (Warburg effect). ENO1 induces hypoxic stress through hypoxia-responsive elements in various cancer cells.<sup>50–54</sup> Cell surface ENOA promotes cancer invasion when subjected to



specific posttranslational modifications, such as acetylation, methylation and phosphorylation.<sup>55</sup>

Kuan-Chung Hsiao et al<sup>56</sup> in their study suggested that  $\alpha$ -enolase has a significant role in cancer metastasis as  $\alpha$ -enolase colocalized with uPA and tPA proteins and was present at the site of pericellular degradation of ECM components. On the other hand, treatment with antibody against  $\alpha$ -enolase in vitro suppressed cell-associated plasminogen and MMP activation, collagen and gelatin degradation and cell invasion. Thus, it was demonstrated that surface  $\alpha$ -enolase promotes ECM degradation and invasion of cancer cells and that targeting surface  $\alpha$ -enolase is a promising approach to suppress tumor metastasis.

### Plasminogen Receptor (KT) (Plg-R(KT))

Plg-RKT is a single 147–amino acid protein with a molecular mass of 17.3 kDa.<sup>57</sup> It is an only integral membrane plasminogen receptor, which contains two transmembrane helices with a C-terminal lysine residue as the integral membrane protein. Studies demonstrated that the expression of Plg-RKT is induced during differentiation of monocytes. It is broadly expressed in small aggregates in human tissues, blood monocytes and lymphocytes but is absent in RBCs and colocalized with the urokinase receptors uPA and tPA. Hence, on the cell surface, Plg-RKT allows plasminogen and uPA to be in close proximity. This mechanism of colocalization of plasminogen, its receptor and two major plasminogen activators illustrates that Plg-RKT regulates plasminogen activation on the cell surface.<sup>37</sup> It is also

involved in chemotaxis and chemokinesis of human monocytes. Plg-RKT also plays an important role in macrophage migration–dependent activation of MMPs.<sup>58</sup> Plg-RKT may be a novel target to plasminogen biology as it is widely expressed on the cancer cells and serves to modulate plasminogen activation.

### Histone H2B

Histone H2B (H2B) is a small and highly conserved protein of 17-kDa MW with a C-terminal lysine. It is primarily found within the eukaryotic cell nucleus. A dimer of histones H2A, H3 and H4, H2B forms the nucleosome core particle, a basic organizational unit of chromosomal DNA. It plays an important role in DNA replication, DNA repair, transcriptional regulation, and maintenance of chromosomal stability. H2B is a membrane protein highly expressed on the cell surface of blood cells and cancer cells and is also involved in the activation of lymphocytes during apoptosis and cancer progression.<sup>59</sup> On the cell surface, H2B is a plasminogen-binding protein and an increased expression of this receptor is correlated with upregulation of the plasminogen-binding capacity of cells.<sup>37</sup> H2B translocates through a calcium regulated mechanism, where it is tethered to the cell surface via an anionic interaction with phosphatidylserine, which plays the role of an anchor to the cell surface.<sup>60</sup> H2B is also involved in the inflammatory response, which enhances expression and binding of plasminogen, generation of plasmin and migration of macrophages to the sites of inflammation.<sup>61</sup> Table 1 and 2 summarises variation

**Table 2.** Comparison between different plasminogen receptors in terms of functions, expression, and clinical correlation.

| PLASMINOGEN RECEPTOR | FUNCTIONS AND EXPRESSION  | CLINICAL CORRELATION              | REFERENCES             |
|----------------------|---|-----------------------------------|------------------------|
| Annexin AII-S100A10  | Involved in various cell functions including angiogenesis, proliferation, apoptosis, cell migration, invasion and adhesion. Up-regulation is seen in pancreatic, colorectal, and brain tumors. Highly expressed in human high grade primary gliomas tissues was directly correlated with advanced clinical stage. mRNA is highly expressed in invasive breast cancer and ductal carcinom and protein levels are high in brain, bone, kidney and lung cancer   | Diagnosis, prognosis and survival | [28], [38], [67], [68] |
| CK8                  | Involved in the signaling pathways involved in cell growth, death and motility. It may affect tumor progression through several signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt, Wnt, and extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK) signaling pathways. CK8 is overexpressed both at the mRNA and the protein level in various carcinomas like breast, colon, gastric, liver and lung which is associated with tumor progression and lower survival | Diagnosis, prognosis and survival | [41], [45], [69]       |
| ENOA                 | It is involved in activation of plasmin, ECM degradation and also supports anaerobic proliferation. Its expression is increased in many tumors like lung, breast and pancreatic. It is associated in cancer progression by contributing to cell invasion, proliferation and metastasis. ENOA is overexpressed both at the mRNA and the protein level in breast, colon, gastric, head and neck, liver, ovarian, pancreatic, prostate and lung cancer   | Prognosis and survival            | [48], [50], [51]       |
| Plg-R(KT)            | It is co-localized with the urokinase receptor, uPA and tPA. It is involved in monocyte/macrophage recruitment, migration and invasion in the pathogenesis of inflammatory and autoimmune diseases  | Prognosis and survival            | [38], [58], [59]       |
| HB2                  | It is mainly associated with inflammatory responses by inducing calcium mobilization through L-type calcium channels leading to H2B translocation on the cell surface where phosphatidylserine acts as an anchor and induces binding of plasminogen followed by activation of plasmin, leading to ECM degradation   | Prognosis and survival            | [38], [60], [62]       |



in properties and functions of the plasminogen receptors discussed in the present review.

## Plasminogen Receptors as Therapeutic Targets in Cancer

Despite heterogeneous numbers of plasminogen receptors existing in eukaryotic cells, only a few receptors such as annexin II–S100A10 heterotetramer, ENOA and CK8 have a renowned role in human cancer progression by promoting plasmin-dependent tumor invasion. These receptors form a multiprotein complex with tPA, uPAR and integrins without interacting with plasminogen activators.<sup>62</sup> They also interact with the actin cytoskeleton and promote the migration of tumor cells independent of plasminogen binding, emphasizing their importance as potential targets for therapeutic purposes.<sup>63</sup> Thus, blocking plasminogen receptors with monoclonal antibodies and DNA-based vaccination or silencing plasminogen receptor genes using siRNA or short hairpin RNA may be a promising strategy to counteract invasion and metastasis.<sup>64</sup> Stereospecific radiation treatment offers a distinct opportunity for temporal and spatial regulation of gene expression at tumor sites by means of inducible promoters. Rao et al<sup>65</sup> confirmed this by the cell death and decrease in migration and invasion by downregulation of uPA and uPAR in meningioma cell lines. Utilization of specific plasminogen receptors to mediate tissue-specific or cell-specific responses is envisioned like annexin II–S100A10 heterotetramer is the major Plg-R on endothelial cells (ECs), H2B, is abundant on monocytoïd cells, integrins and uPAR localizes to the migrating cells. The tissue-specific expression of plasminogen receptors can be explored to target specific plasminogen receptors.<sup>66</sup>

## Acknowledgment

The present review was supported by GITAM University.

## Author Contributions

Analyzed the data: RM. Wrote the first draft of the manuscript: SK. Both the authors contributed to the writing of the manuscript and agree with manuscript results and conclusion, jointly developed the structure and arguments for the paper, made critical revisions and approved final version.

## REFERENCES

1. Ramos-DeSimone N, Hahn-Dantona E, Siple J. Activation of matrix metalloproteinase-9 (MMP9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. *J Biol Chem.* 1999;274:10366–10376.
2. Lopez-Aleman R, Longstaff C, Hawley S, et al. Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-enolase. *Am J Hematol.* 2003;72:234–242.
3. Moller LB. Structure and function of the urokinase receptor. *Blood Coagul Fibrinolysis.* 1993;4:293–303.
4. Lund LR, Green KA, Stoop AA, et al. Plasminogen activation independent of uPA and tPA maintains wound healing in gene-deficient mice. *EMBO J.* 2006; 25(12):2686–2697.

5. Plow EF, Doeuvre L, Da R. So many plasminogen receptors: why? *J Biomed Biotechnol.* 2012;2012:14180–14186.
6. Phipps KD, Surette AP, O'Connell PA, Waisman DM. Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites. *Cancer Res.* 2011;71(21):6676–6683.
7. Dejouvencel T, Doeuvre L, Lacroix R. Fibrinolytic cross-talk: a new mechanism for plasmin formation. *Blood.* 2010;115(10):2048–2056.
8. Kwaan HC, McMahon B. The role of plasminogen-plasmin system in cancer. *Cancer Treat Res.* 2009;148:43–66.
9. Andreasen PA, Egelund R, Petersen HH. The plasminogen activation system in tumor growth, invasion, and metastasis. *Cell Mol Life Sci.* 2000;57:25–40.
10. Dano K, Behrendt N, Hoyer-Hansen G, et al. Plasminogen activation and cancer. *Thromb Haemost.* 2005;93:676–681.
11. McMahon B, Kwaan HC. The plasminogen activator system and cancer. *Pathophysiol Haemost Thromb.* 2008;36:184–194.
12. Das R, Pluskota E, Plow EF. Plasminogen and its receptors as regulators of cardiovascular inflammatory responses. *Trends Cardiovasc Med.* 2010;20(4):120–124.
13. Pluskota E, Soloviev DA, Bdeir K, Cines DB, Plow EF. Integrin  $\alpha$ M $\beta$ 2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. *J Biol Chem.* 2004;279(17):18063–18072.
14. Tang L, Han X. The urokinase plasminogen activator system in breast cancer invasion and metastasis. *Biomed Pharmacother.* 2013;67(2):179–182.
15. Rein-Smith CM, Church FC. Emerging pathophysiological roles for fibrinolysis. *Curr Opin Hematol.* 2014;21(5):438–444.
16. Miles LA, Hawley SB, Baik N, Andronicos NM, Castellino FJ, Parmer RJ. Plasminogen receptors: the sine qua non of cell surface plasminogen activation. *Front Biosci.* 2005;10:1754–1762.
17. Miles LA, Andronicos NM, Baik N, Parmer RJ. Cell-surface actin binds plasminogen and modulates neurotransmitter release from catecholaminergic cells. *J Neurosci.* 2006;26(50):13017–13024.
18. Plow EF, Ploplis VA, Busuttill S, Carmeliet P, Collen D. A role of plasminogen in atherosclerosis and restenosis models in mice. *Thromb Haemost.* 1999;82 (suppl 1):4–7.
19. Plow EF, Das R, Ganapathy S, Settle M. Plasminogen promotes macrophage phagocytosis in mice. *Blood.* 2014;124(5):679–688.
20. Munn DH, Cheung NK. Antibody-independent phagocytosis of tumor cells by human monocyte-derived macrophages cultured in recombinant macrophage colony-stimulating factor. *Cancer Immunol Immunother.* 1995;41(1):46–52.
21. Madureira PA, Surette AP, Phipps KD, Taboski MA, Miller VA, Waisman DM. The role of the annexin A2 heterotetramer in vascular fibrinolysis. *Blood.* 2011;118:4789–4797.
22. Dutta S, Bandyopadhyay C, Bottero V, et al. Angiogenin interacts with the plasminogen activation system at the cell surface of breast cancer cells to regulate plasmin formation and cell migration. *Mol Oncol.* 2014;8(3):483–507.
23. Higazi AA, Finci-Yeheskel Z, Samara AA, Aziza R, Mayer M. Stimulation of plasmin activity by oleic acid. *Biochem J.* 1992;282(pt 3):863–866.
24. Choi KS, Fitzpatrick SL, Filipenko NR, et al. Regulation of plasmin-dependent fibrin clot lysis by annexin II heterotetramer. *J Biol Chem.* 2001;276:25212–25221.
25. Hembrough TA, Li L, Gonias SL. Cell-surface cytoke- ratin 8 is the major plasminogen receptor on breast cancer cells and is required for the accelerated activation of cell-associated plasminogen by tissue-type plasminogen activator. *J Biol Chem.* 1996;271:25684–25691.
26. 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.
27. Andronicos NM, Ranson M, Bognacki J, Baker MS. The human ENO1 gene product (recombinant human alpha-enolase) displays characteristics required for a plasminogen binding protein. *Biochem Biophys Acta.* 1997;1337:27–39.
28. Luo M, Hajjar KA. Annexin A2 system in human biology: cell surface and beyond. *Semin Thromb Hemost.* 2013;39(4):338–346.
29. Das R, Burke T, van Wagoner DR, Plow EF. L-type calcium channel blockers exert an antiinflammatory effect by suppressing expression of plasminogen receptors on macrophages. *Circ Res.* 2009;105:167–175.
30. Kang HM, Choi KS, Kassam G, Fitzpatrick SL, Kwon M, Waisman DM. Role of annexin II tetramer in plasminogen activation. *Trends Cardiovasc Med.* 1999;9:92–102.
31. Shiozawa Y, Havens AM, Jung Y, et al. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem.* 2008;105:370–380.
32. Gerke V, Moss SE. Annexins: from structure to function. *Physiol Rev.* 2002;82: 331–371.
33. Deora AB, Kreitzer G, Jacovina AT, Hajjar KA. An annexin 2 phosphorylation switch mediates p11-dependent translocation of annexin 2 to the cell surface. *J Biol Chem.* 2004;279:43411–43418.
34. Ceruti P, Principe M, Capello M, Cappello P, Novelli F. Three are better than one: plasminogen receptors as cancer theranostic targets. *Exp Hematol Oncol.* 2013; 2(1):12.
35. Swisher JF, Khatri U, Feldman GM. Annexin A2 is a soluble mediator of macrophage activation. *J Leukoc Biol.* 2007;82:1174–1184.



36. Zhang J, Guo B, Zhang Y, Cao J, Chen T. Silencing of the annexin II gene down-regulates the levels of S100A10, c-Myc, and plasmin and inhibits breast cancer cell proliferation and invasion. *Saudi Med J*. 2010;31:374–381.
37. Godier A, Hunt BJ. Plasminogen receptors and their role in the pathogenesis of inflammatory, autoimmune and malignant disease. *J Thromb Haemost*. 2013;11(1):26–34.
38. Sharma M, Blackman MR, Sharma MC. Antibody-directed neutralization of annexin II (ANX II) inhibits neoangiogenesis and human breast tumor growth in a xenograft model. *Exp Mol Pathol*. 2011;92:175–184.
39. Hesse M, Magin TM, Weber K. Genes for intermediate filament proteins and the draft sequence of the human genome: novel keratin genes and a surprisingly high number of pseudogenes related to keratin genes 8 and 18. *J Cell Sci*. 2001;114:2569–2575.
40. Oshima RG, Baribault H, Caulin C. Oncogenic regulation and function of keratins 8 and 18. *Cancer Metastasis Rev*. 1996;15:445–471.
41. Caulin C, Ware CF, Magin TM, Oshima RG. Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. *J Cell Biol*. 2000;149:17–22.
42. Gilbert S, Loranger A, Daigle N, Marceau N. Simple epithelium keratins 8 and 18 provide resistance to Fas-mediated apoptosis. The protection occurs through a receptor-targeting modulation. *J Cell Biol*. 2001;154:763–773.
43. Srikanth B, Vaidya MM, Kalraiya RD. O-GlcNAcylation determines the solubility, filament organization, and stability of keratins 8 and 18. *J Biol Chem*. 2010;285:34062–34071.
44. Hembrough TA, Vasudevan J, Allietta MM, Glass WF, Gonias SL. Cell-surface cytokeratin 8 is the major plasminogen receptor on breast cancer cells and is required for the accelerated activation of cell-associated plasminogen by tissue-type plasminogen activator. *J Cell Sci*. 1995;108:1071–1082.
45. Obermajer N, Doljak B, Kos J. Cytokeratin 8 ectoplasmic domain binds urokinase-type plasminogen activator to breast tumor cells and modulates their adhesion, growth and invasiveness. *Mol Cancer*. 2009;8:88–98.
46. Gonias SL, Hembrough TA, Sankovic M. Cytokeratin 8 functions as a major plasminogen receptor in select epithelial and carcinoma cells. *Front Biosci*. 2001;1(6):1403–1411.
47. Pancholi V. Multifunctional alpha-enolase: its role in diseases. *Cell Mol Life Sci*. 2001;58:902–920.
48. Kato K, Asai R, Shimizu A, Suzuki F, Ariyoshi Y. Immunoassay of three enolase isozymes in human serum and in blood cells. *Clin Chim Acta*. 1983;127:353–363.
49. Royds JA, Parsons MA, Taylor CB, Timperley WR. Enolase isoenzyme distribution in the human brain and its tumours. *J Pathol*. 1982;137:37–49.
50. Warburg O. *The Metabolism of Tumours*. London: Constable; 1930.
51. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324:1029–1033.
52. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res*. 1998;58:1408–1416.
53. Lu Z, Sack MN. ATF-1 is a hypoxia-responsive transcriptional activator of skeletal muscle mitochondrial-uncoupling protein 3. *J Biol Chem*. 2008;283:23410–23418.
54. Sedoris KC, Thomas SD, Miller DM. Hypoxia induces differential translation of enolase/MBP-1. *BMC Cancer*. 2010;10:157–163.
55. Felez J. Plasminogen binding to cell surfaces. *Fibrinolysis Proteolysis*. 1998;12:183–189.
56. Hsiao KC, Shih NY, Fang HL, et al. Surface  $\alpha$ -enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. *PLoS One*. 2013;8(7):e69354.
57. Strickland DK. A new plasminogen receptor. *Inside Blood*. 2010;115:7.
58. Lighvani S, Baik N, Diggs JE, Khaldoyanidi S, Parmar RJ, Miles LA. Regulation of macrophage migration by a novel plasminogen receptor Plg-R KT. *Blood*. 2011;118:5622–5630.
59. Das R, Burke T, Plow EF. Histone H2B as a functionally important plasminogen receptor on macrophages. *Blood*. 2007;110:3763–3772.
60. Das R, Plow EF. Phosphatidylserine as an anchor for plasminogen and its plasminogen receptor, histone H2B, to the macrophage surface. *J Thromb Haemost*. 2011;9:339–349.
61. 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(pt 3):763–769.
62. Capello M, Ferri-Borgogno S, Cappello P, Novelli F. Alpha-Enolase: a promising therapeutic and diagnostic tumor target. *FEBS J*. 2011;278:1064–1074.
63. Liu KJ, Shih NY. The role of enolase in tissue invasion and metastasis of pathogens and tumor cells. *J Cancer Mol*. 2007;3:45–48.
64. Cappello P, Rolla S, Chiarle R, et al. Vaccination with ENO1 DNA prolongs survival of genetically engineered mice with pancreatic cancer. *Gastroenterology*. 2013;144(5):1098–1106.
65. Rao Gogineni V, Kumar Nalla A, Gupta R, et al. Radiation-inducible silencing of uPA and uPAR in vitro and in vivo in meningioma. *Int J Oncol*. 2010;36(4):809–816.
66. Sloand EM, Pfannes L, Scheinberg P, et al. Increased soluble urokinase plasminogen activator receptor (suPAR) is associated with thrombosis and inhibition of plasmin generation in paroxysmal nocturnal hemoglobinuria (PNH) patients. *Exp Hematol*. 2008;36(12):1616–1624.
67. Connell PA, Surette AP, Liwski RS, Svenningsson P, Waisman DM. S100A10 regulates plasminogen-dependent macrophage invasion. *Blood*. 2010;116:1136–1146.
68. Wang CY, Lin CF. Annexin A2: its molecular regulation and cellular expression in cancer development. *Dis Markers*. 2014;2014:308976.
69. Makino T, Yamasaki M, Takeno A, et al. Cytokeratins 18 and 8 are poor prognostic markers in patients with squamous cell carcinoma of the oesophagus. *Br J Cancer*. 2009;101(8):1298–1306.