

## Editorial

# Tissue Plasminogen Activator: Side Effects and Signaling

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## INTRODUCTION

According to the most recent report from American Heart Association, stroke is among the leading causes of death in general population [1]. The predominant form of stroke is the ischemic stroke, which is caused by the clot within a blood vessel that disrupts the blood supply. Currently, the only FDA-approved treatment is tissue-type plasminogen activator (tPA). tPA is a member of the serine protease family that plays a pivotal role in the homeostasis of blood coagulation/fibrinolysis and matrix regulation [2-5]. Generally, tPA converts plasminogen into active plasmin and activates the fibrinolysis process to dissolve the clot and improve or restore the blood supply. However, besides its beneficial protease activities, the clinical application of tPA has been limited by the harmful side effects that not related to its catalytic function [2,6-11]. In addition, although tPA, as a protease, induces matrix degradation, our investigations demonstrate that tPA promotes tissue fibrosis, a disease condition characterized by excessive matrix accumulation [2,12-15].

### The structure and biological function of tPA

tPA is a 69-kDa glycoprotein synthesized within cells and is released as a single chain enzyme. Plasmin subsequently cleaves it into a two-chain form (heavy chain and light chain). There are 4 domains within the single-chain tPA: 1) a finger (F) domain, which is homologous to fibronectin; 2) an EGF domain, which is homologous to EGF; 3) two kringle (K) domains; and 4) the catalytic protease (P) domain. The P domain forms the light chain, while the rest F, EGF, and K domains form the heavy chain. The active site responsible for tPA protease activity consists of Histidine 322, Asparagine 371, and Serine 478 [16]. Mutagenesis analysis indicates that single mutation of Serine 478 to Alanine renders tPA catalytically inactive, while its other function remains intact [17].

Our recent studies demonstrate that tPA is actually a molecule with dual functions [2,12-15]. As a serine protease, tPA plays a pivotal role in the homeostasis of blood coagulation/fibrinolysis and extracellular matrix regulation [2]. As a cytokine, tPA executes multiple actions by binding to its membrane receptors and triggering profound intracellular signaling events [2,12-15,18].

### Signaling of tPA

Although tPA does not have a dedicated and specific receptor, there are at least two known candidates that functionally and

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biologically serve as tPA receptor: LDL receptor-related protein-1 (LRP-1) [19] and annexin A2 [20].

### LRP-1-mediated tPA signaling

LRP-1, also known as  $\alpha$ 2-macroglobulin receptor ( $\alpha$ 2MR) [19] or type V TGF- $\beta$  receptor (T $\beta$ R-V) [21], is a member of the LDL receptor family [22,23]. Mature LRP-1 consists of an extracellular 515-kDa  $\alpha$  subunit and an 85-kDa  $\beta$  subunit with a transmembrane segment and cytoplasmic tail containing two NPxY motifs and numerous tyrosine residues [22,24,25]. tPA has been shown to bind to the domains II and IV in the extracellular region of LRP-1 [22,26].

In the numerous organ injury models including brain, liver, and kidneys, tPA expression is up-regulated [6,12,13,27]. Our recent work demonstrated that myeloid cells are the major source of the endogenous tPA induction in the diseased organs [28]. The myeloid-derived tPA interacts with the LRP-1 on various cells to initiate cell type-specific signaling to modulate cellular processes and cell differentiation. Our previous work demonstrated that LRP-1 on fibroblasts mediates multiple tPA signaling cascades to promote fibroblast activation, transdifferentiation, growth and survival, leading to renal fibrosis: 1) tPA induces matrix metalloproteinases (MMPs) production to initiate the epithelial mesenchymal transition (EMT) through LRP-1-mediated MAPK pathway [13]; 2) tPA promotes the survival, proliferation, and interstitial accumulation of fibroblasts in the diseased kidneys through p90RSK-mediated Bad or GSK3 $\beta$  signaling [2,14]. We also showed that tPA induces the phosphorylation of LRP-1 Tyr 4507, which is indispensable to tPA-mediated fibroblast proliferation [14]; 3) tPA promotes myofibroblast activation by activating LRP-1-mediated  $\beta$ 1 integrin/ integrin-linked kinase (ILK) signaling [12]. In macrophages, Cao, *et al* demonstrated that genetic inactivation of integrin Mac-1, tPA, PAI-1 or LRP-1 abrogates LPS-induced peritoneal macrophage efflux, and tPA forms complex with LRP-1, Mac-1, and PAI-1 to promote macrophage migration [29]. We recently found that LRP-1 mediates tPA-induced macrophage motility through activation of FAK and Rac-1 signaling [28]. In the brain, LRP-1-mediated MMP-9 induction in human cerebral microvascular endothelial cells has been considered as one of the cellular mechanisms of tPA-induced neurotoxic side effects [30], tPA has been shown to induce cerebral vascular LRP-1-mediated opening of blood-

brain barrier [6], and tPA and LRP-1 have been shown to mediate ischemia-induced NF- $\kappa$ B activation [31]. tPA also activates Rac1 and controls LRP-1-mediated Schwann cells migration [32]. In neurons, LRP-1 mediates tPA-induced Trk receptor phosphorylation and activation of downstream Akt and Erk pathway, leading to neurite outgrowth [18].

### A2-mediated tPA signaling

Annexin A2 is a member of the Ca<sup>2+</sup>- and phospholipid-binding protein family. Annexin A2 has been identified as a major membrane receptor of tPA on endothelial [33], microglia cells [34], and other cancer cells [35]; and is implicated in mediating certain signal transductions [9,34,36]. tPA has been shown to bind to the hexapeptide LCKLSL (residues 7–12) in the N terminus of annexin A2 [37]. Recently, we demonstrated that tPA induces the aggregation of annexin A2 and integrin CD11b on macrophages and the subsequently activation of ILK pathway, leading to NF- $\kappa$ B activation [15]. Activation of NF- $\kappa$ B signaling contributes to tPA-mediated macrophage migration [28]. In addition, the finger domain of tPA has been shown to bind to annexin A2 and induce microglial activation to cause brain injury [20]. Annexin A2 on the pancreatic cancer cells has also been shown to be responsible for tPA-induced cell proliferation [9].

### FUTURE PERSPECTIVE

Although the direct *in vivo* investigations towards tPA cytokine functions are still missing, numerous studies clearly indicate that the side effects and toxicity of tPA are likely mediated by its protease-independent cytokine functions. Previous report that plasminogen activator inhibitor type 1 (PAI-1)-derived hexapeptide (EEIIMD) blocks tPA signaling and reduces the neurotoxic effects of tPA without compromising its fibrinolytic activity [11] strongly supports the above view, and lays a strong foundation for future development of therapeutic strategies targeting tPA side effects and toxicity.

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