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Beyond Clot Dissolution; Role of Tissue Plasminogen Activator in Central Nervous System

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Abstract – Tissue plasminogen activator (tPA) is a serine protease catalyzing the proteolytic conversion of plasminogen into plasmin, which is involved in thrombolysis. During last two decades, the role of tPA in brain physiology and pathology has been extensively investigated. tPA is expressed in brain regions such as cortex, hippocampus, amygdala and cerebellum, and major neural cell types such as neuron, astrocyte, microglia and endothelial cells express tPA in basal status. After strong neural stimulation such as seizure, tPA behaves as an immediate early gene increasing the expression level within an hour. Neural activity and/or postsynaptic stimulation increased the release of tPA from axonal terminal and presumably from dendritic compartment. Neuronal tPA regulates plastic changes in neuronal function and structure mediating key neurologic processes such as visual cortex plasticity, seizure spreading, cerebellar motor learning, long term potentiation and addictive or withdrawal behavior after morphine discontinuance. In addition to these physiological roles, tPA mediates excitotoxicity leading to the neurodegeneration in several pathological conditions including ischemic stroke. Increasing amount of evidence also suggest the role of tPA in neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis even though beneficial effects was also reported in case of Alzheimer's disease based on the observation of tPA-induced degradation of Aβ aggregates. Target proteins of tPA action include extracellular matrix protein laminin, proteoglycans and NMDA receptor. In addition, several receptors (or binding partners) for tPA has been reported such as low-density lipoprotein receptor-related protein (LRP) and annexin II, even though intracellular signaling mechanism underlying tPA action is not clear yet. Interestingly, the action of tPA comprises both proteolytic and non-proteolytic mechanism. In case of microglial activation, tPA showed non-proteolytic cytokine-like function. The search for exact target proteins and receptor molecules for tPA along with the identification of the mechanism regulating tPA expression and release in the nervous system will enable us to better understand several key neurological processes like learning and memory as well as to obtain therapeutic tools against neurodegenerative diseases.

Key words □ synaptic plasticity, ECM, dendrite, neurotoxicity, Alzheimer's disease, ischemia

INTRODUCTION

Plasminogen activator and plasminogen system is primarily involved in the regulation of thrombolysis, i.e. blood clot dissolution. Plasminogen activator is a serine protease which cleaves substrates such as plasminogen. The cleavage of plasminogen yields another potent protease called plasmin which shows strong fibrinolytic activity. There are two types of plasminogen activator: urokinase type and tissue type. In CNS, tissue type plasminogen activator mediates the cleavage of plasminogen, laminin and NMDA receptor among others and regulates diverse array of neural processes ranging from neural development to neurodegeneration. In addition, it is clear that tPA possesses nonproteolytic neuromodulator- and cytokine-like function which makes it more intriguing than a simple protease. Clinically, tPA is the only FDA-approved therapeutic reagent in embolitic stroke. However, the use of tPA in stroke patient has limitations such as relatively short time window as well as the much debated neurotoxic side effect. Therefore, understanding

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the function as well as the regulatory mechanism governing tPA release and expression in CNS is important not only for the neurobiological aspects but also in clinical situations. In this review, we will address the recent progress in the role of tPA in neuro-physiological and neuro-pathological situations

Localization of tPA in central nervous system

In brain, tPA is found in almost all regions with highest expression in cerebellum, hippocampus, cortex and amygdala. tPA is expressed in all major cell types found in CNS including neuron, astrocytes, microglia and endothelial cells (Tsirka *et al.*, 1996, 1997; Seeds *et al.*, 1995; Kalderon *et al.*, 1990; Kim *et al.*, 2003). The subcellular distribution of tPA in neural cells is not clear even though it is generally believed that tPA is enriched in neural growth cone (Garcia *et al.*, 1994; Pittman *et al.*, 1989) during development and released from axon terminal upon neural activity (Baranes et al., 1997). The localization of tPA on growth cone is well suited with its proposed role in neural development and axonal elongation presumably by degrading extracellular matrix components such as laminin and proteoglycan. In neuroendocrine cell line AtT20, exogenously expressed tPA is localized in dense core granule (Santell *et al.*, 1999). In this study, stimulation with 8-bromo-cAMP, the secretagogue which promotes the release of dense granule contents increased the release of tPA. In addition, tPA was colocalized with adrenocorticotropic hormone (ACTH), an endogenous protein that is stored in dense core granules. More recently, using recombinant tPA tagged with green fluorescent protein, Scalleter group have reported that tPA is localized and transported in dense core granules and released by exocytosis (Lochner *et al.*, 1998; Silverman et al., 2005). Interestingly, recent evidences are suggesting that tPA is localized in neuronal dendrite as well (Silverman *et al.*, 2005; Shin *et al.*, 2004). Investigating the contribution of dendritic (postsynaptic) tPA on the regulation of neuronal function such as its role in BDNF signaling is one of the active areas of research in this field.

Regulation of tPA release and expression

1) The release of tPA

Even though the existence of tPA in regulated secretory pathways has been reported in neuroendocrine cells such as PC12 and AtT20 as well as in cultured neuron (Palmer *et al.*, 1997; Santell et al., 1999; Silvermann *et al.*, 2005), the mechanism(s) regulating tPA release are poorly understood. In the pseudoneuronal cell line PC12, tPA is released following KCl (60 mM) stimulation, a process dependent upon calcium entry into the cell (Gualandris *et al.*, 1996). Others have shown that tPA release from mouse cortical neurons is dependent upon NMDA signaling (Nicole *et al.*, 2001).

Regarding the mechanism of neuropeptide release from the neuron, relatively extensive works have been reported with several growth factors focusing on BDNF. Both axonal (Kohara *et al.*, 2001) and dendritic (Goodman *et al.*, 1996; Horch and Katz, 2002; Wu *et al.*, 2004) release of BDNF has been reported. Interestingly, dendritic release of BDNF from hippocampal neuron was modulated by group I mGluR via PLC signaling pathways (Canossa *et al.*, 2001; Balkowiec and Katz, 2002) and probably by intracellular calcium store (Griesbeck *et al.*, 1999). It has been also reported that hypothalamic oxytocin neurons release oxytocin from dendrite, which is regulated by intracellular calcium store (Ludwig *et al.*, 2002) and physiological signals such as changes in female reproductive cycle (de Kock *et al.*, 2003). Interestingly, Morsette et al. also reported that group I mGluR regulates oxytocin and vasopressin release from explants of the hypothalamoneurohypophysial system (Morsette *et al.*, 2001). Recently, the existence of tPA in dendrite was also reported (Silverman *et al.*, 2005; Shin *et al.*, 2004) and Scalleter group reported the dendritic release of transfected tPA (Lochner *et al.*, 2006). Even though the release of tPA from dense core granule pathway is most plausible mechanism regulating tPA release from axonal terminal, these results imply that mGluR-dependent dendritic release may be one of the general mechanisms regulating neuropeptide release from neurons, which needs further verification in the future.

2) tPA expression

a. transcriptional control

In normal circumstances, tPA is expressed at relatively low level in several discrete regions of CNS. However, the level of tPA is rapidly increased after neural stimulation protocols including motor learning, seizure and long-term potentiation (Qian *et al.*, 1993; Carroll *et al.*, 1994; Seeds *et al.*, 1995). In a study designed to identify target genes upregulated as immediate early genes by neuronal activity, Qian et al. found tPA behaves as an immediate early gene after several different kinds of neural stimulation protocols (Qian *et al.*, 1993). Most importantly, brief high-frequency stimulation of the perforant path that produces long-term potentiation (LTP) causes an

NMDA (*N* -methyl-D-aspartate) receptor-mediated increase in the levels of tPA mRNA which is restricted to the granule cells of the ipsilateral dentate gyrus (Qian *et al.*, 1993). Similarly, in rats trained for a complex cerebellum-dependent motor task, tPA mRNA expression was increased in the Purkinje neurons within an hour as determined by *in situ* hybridization as well as the expression of tPA protein (Seeds *et al.*, 1995). Regarding the promoter activity regulating the transcriptional activity of tPA gene, Carroll et al. characterized that 5'-flanking region of tPA is important in the transcriptional regulation of tPA expression during CNS development (Carroll *et al.*, 1994). More recently, Dr. Medcalf group more thoroughly characterized the promoter activity of tPA genes and demonstrated that a 9.5 kb fragment of tPA promoter regions dictate the regional expression of tPA in the brain and contains regulatory sites for several transcription factors including AP-1 (Yu *et al.*, 2001). These results suggest that the transcription of tPA is regulated following physiological and pathological neural stimulation and several transcription factors including AP-1 and NF-κB regulate the induction of tPA.

b. translational control

Recently, it has been reported that the expression of tPA also might be under translational control at activated synapse (Shin et al., 2004). The mRNA encoding tPA is localized in synaptic regions of cultured hippocampal neuron and is relatively enriched as compared with general mRNA population. In intact and isolated synapse preparation called synaptoneurosome, stimulation of metabotropic glutamate receptor (mGluR) mediates rapid polyadenylation of tPA mRNA followed by increased expression of tPA protein presumably by increased translational efficiency conferred by longer poly A tail of tPA mRNA. In oocytes, tPA protein synthesis is regulated by mRNA polyadenylation, although the exact mechanism regulating polyadenylation of tPA mRNA in oocytes is not known (Huarte *et al.*, 1987). The 96 nucleotides upstream of the hexanucleotide poly A signal sequence in the tPA 3'UTR sequence has consensus sites (UUUUAAU--27nt--UAUUUUAAU--53nt--AAUAAA--; accession J03520) for the binding of an RNA binding protein called CPEB (Cytoplasmic Polyadenylation Element Binding Protein), which regulates the polyadenylation and translation of target mRNA such as α-CAMKII. Co-immunoprecipitation experiments using an antibody against CPEB revealed that tPA mRNA is physically associated with CPEB in cultured hippocampal neuron, which suggest that CPEB may regulates the translation of tPA mRNA by regulating the polyadenylation status (Shin *et al.*, 2004). The rapid upregulation of tPA expression in synaptic regions would enable the local increase in the concentration of tPA followed by the inactivation of tPA activity through the association of inhibitors of tPA such as neuroserpin, which has clear benefit of more sophisticated spatial and temporal control of tPA action in CNS. In case of vasopressin, localization of its mRNA and translational control to maintain the release from dendrite has been relatively well studied (for a review, see Mohr *et al.*, 2002, 2004). These results raise the possibility that the rapid translational control of neuropeptide expression in dendrite (and possibly in postsynaptic regions) is a general mechanism governing the localized action of neuropeptides, which should be verified experimentally in the future.

The role of tPA in neural development

In an early study using NG108 neuroblastoma cells, Krystosek and Seeds (1981, 1986) observed the deposition of tPA in extracellular matrix along the pathway of growth cone movement suggesting an essential role of tPA during neural differentiation. Similarly, tPA expression was regulated by protein kinase A dependent pathway and was essential in neurite extension from PC 12 cell line (Leprince *et al.*, 1991, 1996). Garcia-Rocha and collegues biochemically isolated growth cone fraction from rat brain and they found tPA is a main plasminogen system associated with growth cone fraction and is relatively enriched in the fraction (Garcia-Rocha *et al.*, 1991). In an effort to demonstrate the essential role of tPA in neurite extension, Pittman et al. overexpressed tPA in neuronal cell line PC12 and found the increased neurite forming activity in transfected cells, which correlates well with the level of expression of tPA as well as the increased plasmin-dependent digestion of extracellular matrix (Pittman and deBenedetto, 1995). The role of tPA in axonal growth was best exemplified by the work of Baranes *et al.* (1998). They found that addition of tPA to hippocampal neuron promotes axonal sprouting and concluded tPA plays essential role in neural stimulation dependent outgrowth of hippocampal mossy fiber pathway (Baranes *et al.*, 1998).

Several researchers tried to find out the evidence that tPA is involved in neural cell differentiation and development. Two groups have reported that tPA mRNA is differentially expressed in major cerebellar cell types including Purkinje cells and granule neurons in developmentally-regulated fashion even though they can not find the evidence of the involvement of proteolytic activity in granule cell migration (Ware *et al.*, 1995; Friedman and Seeds, 1995). More direct evidences have been obtained

from the studies using transgenic mice. Seeds et al. reported that in mice lacking tPA, two-fold more migrating cells were observed during the most active periods of granule cell migration, suggesting the positive role of tPA in the regulation of cerebellar granule cell migration (Seeds *et al.*, 1999).

The activity of tPA also plays important role in regeneration of neurite after peripheral nerve injury. Siconolfi et al. have reported that tPA expression is induced after mechanical sciatic nerve injury (Siconolfi *et al.,* 2001a). Interestingly, mice lacking tPA gene showed reduced functional recovery after sciatic nerve crush (Siconolfi *et al.*, 2001b), which suggests that tPA regulates regeneration process as well as axonal development. Extracellular matrix protein components in CNS including chondroitin sulfates proteoglycan, has been suggested to be major impedance for the efficient neural regeneration. Laminin and proteoglycans are among the targets of tPA enzymatic actions (Chen and Strickland, 1997; Nakagami *et al.*, 2000; Wu *et al.,* 2000), and whether tPA activity can regulate the regeneration of injured neuron in CNS remains to be an open question.

The role of tPA in synaptic plasticity

After neural stimulation inducing long-term potentiation (LTP) in neural circuit, tPA behaves as an immediate early gene: i.e. the expression of tPA is rapidly increased in an hour or so (Qian *et al.,* 1993). The involvement of tPA in the regulation of synaptic plasticity has been supported by dozens of studies. In hippocampal mossy fiber pathway, tPA was involved in late-phase LTP as well as the structural remodeling of the pathway (Baranes *et al.*, 1997). The involvement of increased tPA activity in the formation of perforated synapses in cultured hippocampal neuron was also suggested (Neuhoff *et al.,* 1999). Either inhibition of tPA activity or knockout of tPA expression inhibited LTP and learning (Frey *et al.,* 1996; Huang *et al.*, 1996). In contrast, the delivery of tPA into the brain of tPA knock-out mice restored the impaired learning ability (Pawlak *et al.*, 2002). Likewise, the over-expression of tPA in transgenic mice showed increased hippocampal LTP as well as superior hippocampus-dependent spatial orientation learning tasks such as Morris water maze (Madani *et al.,* 1999).

Altered LTP or long term depression (LTD) in tPA disrupted animal was also observed in brain regions other than hippocampus. Centonze et al. reported that LTP induction in corticostriatal pathway, an NMDA receptor-mediated phenomenon, was abolished in tPA knockout animals (Centonze *et al.*, 2002). Using differential display technique, Napolitano et al. reported that tPA gene expression is upregulated after LTD induction in rat striatum (Napolitano *et al.*, 1999) suggesting tPA might be involved in the microstructural rearrangement after LTD induction in striatum. Actually, tPA knockout mice showed altered two-way active avoidance performance, a striatum dependent learning task (Calabresi *et al.*, 2000).

Another brain region extensively studied for the tPA dependent functional and structural plasticity is amygdala. Amygdala is an important brain region regulating stress-response, anxiety behavior and emotional control. Using restrain-induced anxiety behavior as a model system, Pawlak and collegues reported that stress increased tPA expression in amygdala and tPA gene knockout eliminated stress-induced anxiety behavior (Pawlak *et al.*, 2002). In a related study, they found that tPA expression is changed after ventricular injection of stress response regulating hormone, corticotrophin-releasing factor (CRF) (Matys *et al.*, 2004), which might be related to the induction of c-fos, a well known marker of neural activation. More recently, it has been reported that tPA regulates stress-induced spine loss in amygdala (Bennur et al., 2007), which suggests that structural changes induced by tPA plays an important role in the regulation of amygdala-dependent behavioral plasticity. In cerebellum, increased tPA activity was observed after several rounds of cerebellar motor learning task in rats and the ability to learn to walk across over irregularly spaced pegs was severely impaired in tPA deficient mice (Seeds *et al.*, 1995, 2003).

The role of tPA in synaptic plasticity was also studied in visual cortex. The development of mammalian visual system depends on the experience-dependent reorganization of visual cortex to respond only to selective subsets of visual inputs, usually represented by a single eye. The reorganization (ocular dominance) happens only during certain period of development, which is called critical period. The deprivation of visual input during the critical period eliminates the ability of the visual cortex to form ocular dominance. Mataga et al. reported that the expression of tPA mRNA was increased in the visual cortex of kitten when stimulated to form ocular dominance plasticity by the injection of l-threo-3,4-dihydroxyphenylserine (Mataga *et al.*, 1996). The infusion of plasminogen activator inhibitor-1 (PAI-1) into the ventricle of a kitten abolished the expression of ocular dominance plasticity suggesting an active role of tPA in the regulation of visual cortical plasticity. In a related study, Mataga et al. also reported that the loss of responsiveness to the deprived eye following monocular deprivation was suppressed in tPA knockout mice. The loss of plasticity was restored by tPA gene dosage-related fashion demonstrating the essential role of tPA in this type of neural plasticity (Mataga

et al., 2002). The same group reported that tPA is involved in visual cortical experience-dependent pruning of dendritic spine, which connects tPA dependent changes in synaptic structure with functional changes in neural activity (Mataga *et al.*, 2004). In a different experimental setting, Muller and Griesinger observed that the reorganization of visual cortical pathways by opening previously closed eyes and closing previously opened eye (reverse occlusion plasticity), was prevented by the inhibition of tPA-plasmin proteolytic pathway (Muller and Griesinger, 1998).

Another example of tPA-dependent plastic changes in brain function can be found from addiction study. Chronic ethanol consumption upregulates NMDA receptor-mediated signaling in brain, which causes brain damages and seizures upon ethanol withdrawal. Pawlak et al. showed that tPA is associated with NR2B subunit of NMDA receptor and is essential for the ethanol-mediated induction (Pawlak *et al.*, 2005). In mice deficient with tPA or plasminogen, the ethanol-induced upregulation of NMDA receptor is prevented resulting in the inhibition of ethanol withdrawal-induced seizures. Interestingly, tPA mediated increase in seizure after ethanol withdrawal was inhibited with NR-2B specific NMDA receptor antagonist, ifenprodil. In nucleus accumbens, tPA/plasmin system is involved in the morphine-dependent dopamine release and hyperlocomotion (Nagai *et al.*, 2004). Single administration of morphine induced tPA expression from nucleus accumbens and repeated treatment abolished this effect. In tPA or plasminogen knockout mice, morphine dependent hyperlocomotion and dopamine release was inhibited and infusion of tPA reinstated the morphine-induced behavioral changes. In addition, tPA deficient mice showed reduced morphine-induced reinforcement (Yan *et al.*, 2007) suggesting that tPA could be a potential target of drug intervention for the misuse of drugs of abuse.

Even though the vast array of data indicating the essential role of tPA in the regulation of neural plasticity, relatively little is known about the cellular mechanism underlying tPA-dependent plasticity control. Low-density lipoprotein (LDL) receptor-related protein (LRP) is a multifunctional endocytic receptor that is expressed abundantly in neurons. Perfusion of receptor-associated protein (RAP), an antagonist for ligand interactions with LRP, into hippocampal slice significantly reduced exogenous tPA-mediated increase in late-phase LTP (L-LTP), which suggests the critical role of LRP in tPA-mediated control of LTP (Zhuo et al., 2000). NMDA receptor-mediated signaling has been reported essential in the regulation of hippocampal LTP induction and it was reported that tPA increased NMDA signaling by cleaving N-terminal region of NR1 subunit (Nicole *et al.*, 2001) even though the involvement of plasmin proteolytic system in this case was questioned by others. More specifically, Arg260 residue at the N-terminal region of NR1 subunit has been suggested to be responsible for the tPA/plasmin-mediated increase in NMDA signaling (Fernandez-Monreal *et al.*, 2004).

Extracellular matrix protein was also suggested as a target of tPA-mediated regulation of neural plasticity. Using organotypic hippocampal slice culture, Nakagami et al. reported that degradation of laminin by plasmin-dependent mechanism is essential for the manifestation of hippocampal LTP (Nakagami *et al.*, 2000).

It has been appreciated for a decade that BDNF is involved in the regulation of synaptic plasticity. Fiumelli et al. reported that BDNF treatment increased tPA expression from cortical neuron (Fiumelli *et al.*, 1999). Alternatively, tPA cleaved proBDNF into its mature form mBDNF. The application of mBDNF increased LTP in tPA or plasminogen-deficient mice suggesting the essential role of tPA/BDNF system in the regulation of hippocampal LTP (Pang *et al.*, 2004). BDNF has been suggested to be effective in the treatment of major depression. Considering the effects of tPA on the BDNF maturation, it is an interesting question whether tPA can be effective in the regulation of depression. Recently, it has been hypothesized that statins, which can regulate tPA expression, may be effective in the treatment of depression through the regulation of tPA/BDNF system, which should be verified experimentally in the future (Tsai, 2006). These studies might add an additional level of importance in understanding the mechanism of tPA-dependent regulation of neural plasticity.

Two edged sword: the role of tPA in neurotoxicity

Considering the proteolytic activity of tPA, it is not hard to imagine that excessive generation of tPA in the brain might be toxic to brain. In a mouse model of excitotoxicity, Tsirka et al. found that mice deficient either tPA or plasminogen are resistant to hippocampal excitotoxicity (Tsirka *et al.*, 1995, 1997). After neural stimulation, the expression of tPA is increased and is related to the increased neurite generation and migration also suggesting the role of tPA in plastic reorganization of neural connection after stimulation. Currently, it is generally assumed that the excessive production of tPA and plasmin degrades target molecules such as laminin leading to the neuronal cell death (Chen and Strickland, 1997; Nagai *et al.*, 1999). In case of laminin, the degradation of laminin along with the excitotoxic-

ity contributes to the cell death by anoikis mechanism. Recently, it has been suggested that laminin-10 is a major subtype degraded by tPA system, which mediates tPA-mediated excitotoxic injury (Indyk *et al.*, 2003).

Another obvious mediator of tPA-induced neurotoxicity is the activation of NMDA receptor. Nicole and colleagues suggested that tPA/plasmin can cleave N-terminal portion of NMDA-receptor, which results in increased activation of the receptor (Nicole *et al.*, 2001).

After excitotoxic injury, microglial cells are activated and produce excess amount of tPA. When the activation of microglial cells are prevented by the infusion of macrophage inhibitory factor (MIF), excitotoxicity-induced increase in microglial activation and tPA release was blocked leading to the prevention of neuronal death (Rogove and Tsirka, 1998). Using cell type-specific overexpression system in tPA knockout mice, Tsirka group demonstrated that neuronal tPA is protective but microglial tPA is detrimental for neuronal survival (Siao *et al.*, 2003).

Even though the majority of the data is indicating the neurotoxic effects of tPA it should be also remembered that tPA showed proteolysis-independent neuroprotective effects at least in one study (Kim *et al.*, 1999). It is possible that the final neurotoxicological outcome is dependent on the several factors including the source of tPA, local concentration as well as the particular experimental paradigms used for the study. In ischemic stroke, tPA is the only FDA-approved pharmacological agent for the treatment of stroke. However, mainly due to the possible neurotoxic effects of tPA as well as the limited time window of application, the use of tPA in ischemic stroke has been controversial. Understanding the neurotoxic mechanism induced by tPA will make the clinical outcome more predictable.

The role of tPA in microglial activation

In addition to the increased production of tPA from activated microglia, it has been also suggested that tPA can activate microglial cells, which may form feed forward activation loop for microglial cells. Interestingly, the activation of microglial cells by tPA does not require proteolytic activity of tPA; i.e. neither the inhibition of tPA proteolytic activity nor the use of non-proteolytic recombinant tPA prevented the induction of microglial activation (Rogove *et al.*, 1999). It is suggested that the finger domain of tPA is necessary for the activation of microglial cells and annexin II acts as a binding partner for tPA on microglial cell membrane (Siao and Tsirka, 2002). Another neural protease thrombin also showed similar non-proteolytic cytokine-like function for microglial activation and it should be investigated further whether the cytokine-like functions of neural proteases are part of general theme in this family.

The role of tPA in neurological diseases.

Due to the role of tPA in synaptic reorganization and neural differentiation as well as neurotoxicity and neuroinflammation, tPA has been implicated in diverse forms of neurological diseases. First, the expression of tPA is increased after various forms of neural stimulation protocol leading to the manifestation of seizure including kainic acid injection and induction of kindling. The increased expression of tPA has been suggested to be responsible for the seizure spreading (Yepes *et al.*, 2002). Interestingly, the seizure spreading effect of tPA was plasminogen-independent suggesting the direct role of tPA. Second, tPA has been implicated in various forms of neurotoxicity including excitotoxic injury and stroke. After transient ischemia, up-regulation of tPA expression has been observed both *in vivo* and *in vitro* (Nagai *et al.*, 2001; Hosomi *et al.*, 2001). In ischemic rats, tPA mediated heparin-induced hemorrhage, which is dependent on the induction of matrix metalloproteinase 9 (MMP-9), an important regulator of neurovascular integrity in ischemic condition (Zhao *et al.*, 2004). The direct role of tPA in the regulation of the permeability of brain microvasculature was also reported. Polavarapu et al. reported that tPA-induced shedding of LRP from astrocyte contributes to the increased permeability of blood vessel (Polavarapu *et al.*, 2006). The involvement of tPA/plasminogen system in the delayed neurotoxicity after transient focal ischemia was also reported (Takahashi *et al.*, 2005). However, care should be given in the interpretation of the experimental data. In ischemic stroke, tPA is used as a thrombolytic with preferential clinical outcomes. Actually, in a carefully designed experimental setting, Klein and colleagues did not find any adverse effect of exogenously administered tPA on ischemic outcomes of both global and focal ischemia (Klein *et al.*, 1999).

The role of tPA in Alzheimer's disease has been appreciated by several researchers. It has been reported that Aβ is physically associated with plasminogen and can activate tPA. Upon activation, tPA/plasmin system can release neuroendocrine factors including chromogranin and VGF, which might have some role in the regulation of neurotransmitter release and apoptotic neuronal cell death (Kranenburg *et al.*, 2005). More recently, the activation of Erk1/2 pathway by tPA has been reported, which mediates Aβ-induced neurotoxicity (Medina *et al.*,

2005). In this case, tPA mediates Erk activation through the regulation of NMDA, PKC and glycogen synthase kinase (GSK) pathways. Alternatively, several researchers reported that tPA mediates plasmin-dependent inhibition of Aβ neurotoxicity as well as the deposition of Aβ aggregates, possibly by degrading Aβ aggregates (Melchor et al., 2003; Tucker et al., 2000a, 2000b).

Multiple sclerosis has also been implicated in tPA-induced neurotoxicity. Aberrant regulation of tPA activity was related to the neuroinflammation and pathophysiology of axonal death in experimental allergic encephalomyelitis (Lu *et al.*, 2002; East *et al.*, 2005; Gveric *et al.*, 2005). The increasing body of evidences indicating the involvement of tPA system in the pathophysiology of several neurological diseases indicates the importance of developing selective pharmacological agents designed to regulate tPA/plasmin activity in the brain as a potential therapeutic targets against neurological diseases.

tPA, unresolved questions

1) tPA receptor

As described above, tPA can bind to several proteins including NMDA receptor, annexin II and LRP. However, it is not surprising to expect the existence of additional receptive proteins in the brain. Recently, it has been suggested that protease activated receptor-1 (PAR-1) is involved in the tPA/plasmin-mediated neural damage in transient focal cerebral ischemia (Junge *et al.*, 1993) as well as the regulation of hyperlocomotion and dopamine release induced by morphine administration (Ito *et al.*, 2007). Exogenous tPA and plasmin directly activated PAR-1 signaling (Junge *et al.*, 203) and the microinfusion of PAR-1 antagonist blocked the plasmin-induced reversal of the inhibition of morphine-mediated dopamine release in tPA knockout mice, which suggest that PAR-1 could be the target of tPA/plasmin action in brain. Active search for true tPA receptor would shed light on the understanding of intracellular signaling mechanisms regulating tPA action in the nervous system.

2) Target proteins for tPA action

Several members of extracellular matrix proteins such as laminin act as tPA substrates. It has been also suggested that tPA can cleave NMDA receptor, which results in increased activity. The list of tPA substrates is still increasing including a recent member, Aβ. Proteomic identification approach will be best suited for the purpose, especially using synaptic or ECM compartments given that the role of tPA in the regulation of ECM and synaptic plasticity.

3) The mechanism of tPA release

Neural activity promotes the release of tPA, presumably from axon terminal (Gualandris *et al.*, 1996). Neural stimulation including NMDA treatment also increased extracellular tPA suggesting the possible release of tPA from dendritic side. The dendritic localization and release from dense core granule pathway has been reported using fluorescence-tagged recombinant tPA (Lochner *et al.*, 2006). However, the exact cellular mechanism regulating tPA release is still unclear. Recently, Tsirka group reported that phospholipase D-1 (PLD-1) regulates tPA release, which is important in the neurite outgrowth in the hippocampus (Zhang *et al.*, 2005). Currently, no recycling mechanism has been reported for the salvage of released tPA, even though massive retrograde movement of tPA puncta can be seen after transfection of recombinant tPA (Lochner et al., 1998). Whether those puncta represents recycled tPA, which can be released upon appropriate stimulus, remains to be an open question.

4) tPA in stem cell biology

The involvement of tPA on neuronal differentiation and regeneration is relatively well known and the reorganization of extracellular matrix is expected to underlie tPA-mediated regulation of synaptic plasticity. One of the questions arising from this observation is whether tPA/plasmin system is involved in the differentiation and migration of stem cell in the nervous system. In a nervous Purkinje neuron defect of cerebellum, the over-expression of tPA mRNA, which causes mitochondrial defect and cell death, was diminished by the transplantation of neural stem cell suggesting the developmental regulation of tPA expression (Li *et al.*, 2006). In cultured adipocyte system, Liang *et al.* observed that PAI-1 modulates differentiation of adipocyte suggesting the involvement of plasmin and ECM reorganization in adipocyte differentiation. Exploring whether tPA is involved in the differentiation and migration of neural stem cell during normal development and neural injury awaits further investigation.

Concluding remarks

In addition to the classical role in thrombolysis, tPA is involved in a diverse array of neurological and pathological phenomena ranging from synaptic plasticity to neurodegenerative diseases. The function of tPA is regulated both by protease-dependent and -independent mechanism. Understanding the physiological and pathological role of tPA in the nervous system will help to pave the way to the better control of key neu-

rological processes such as learning and memory, which requires plastic changes in neural function and interconnection, not to mention the use of tPA as a 'clot buster'.

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