NEURAL REGENERATION RESEARCH
Volume 7, Issue 2, January 2012
www.nrronline.org

Cite this article as: Neural Regen Res. 2012;7(2):146-151.

Tissue plasminogen activator-independent roles of neuroserpin in the central nervous system★

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Abstract

A number of studies have confirmed the existence of tissue-type plasminogen activator-independent roles of neuroserpin, a member of the serine protease inhibitor superfamily. In this review article, we aim to clarify this role. These unique roles of neuroserpin are involved in its neuroprotective effect during ischemic brain injury, its regulation of tumorigenesis, and the mediation of emotion and cognition through the inhibition of urokinase-type plasminogen activator and fibrinolysin, modification of Th cells, reducing plaque formation, promoting process growth and intracellular adhesion, and altering the expression of cadherin and nuclear factor kappa B.

Key Words: neuroserpin; tissue-type plasminogen activator; serpin; cerebral ischemia; tumor; familial encephalopathy with neuroserpin inclusion bodies

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Received: 2011-09-18
Accepted: 2011-11-19
(N20110712001/WLM)

Ma J, Tong Y, Yu D, Mao M. Tissue plasminogen activator-independent roles of neuroserpin in the central nervous system. Neural Regen Res. 2012;7(2):146-151.

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doi:10.3969/j.issn.1673-5374.2012.02.012

INTRODUCTION

Neuroserpin, or proteinase inhibitor 12 (gene symbol SERPINI1), was first found in cultured chicken neuronal axons and is predominantly expressed in human neurons of both the central and peripheral nervous systems[1-2]. Sequence analysis has shown that neuroserpin is a new member of an old proteinase family, the serine proteinase inhibitor (serpin) superfamily[3]. The expression of neuroserpin is mainly induced by neuronal depolarization[4]. A high neuroserpin expression level is detected during the late stage of neuronal development throughout the central nervous system (CNS); however, it is only detected in some regions of the adult brain, such as the hippocampus, hypothalamus, cerebellum, amygdala and sympathetic nerves[3, 5-6], where tissue-type plasminogen activator (tPA) mRNA and/or protein has also been found, during the early stage of neurogenesis[7-8]. The spatial and temporal analysis of neuroserpin and tPA expression suggests a role for neuroserpin in maintaining the proteolytic balance in the CNS, which is crucial for axonogenesis, synaptic connection, and synaptic plasticity[3, 7-8]. The co-expression and preferential interaction with tPA indicates that neuroserpin is a selective inhibitor of tPA in the CNS. The active balance and interaction between these two proteinases are very important for both physiological and pathological events. Under normal conditions, neuroserpin and tPA both participate in processes such as learning, memory, behavior and the regulation of permeability between vascular compartments and the nervous system[8-11]. During pathological courses, such as neurodegeneration, cerebral ischemia and seizures, neuroserpin displays neuroprotective effects by eliminating tPA deleterious proteolytic activity[12-14]. However, many members of the serpin superfamily have shown nonproteinase-inhibitory roles in many processes, such as hormone transport, protein folding, tumor progression, chromatin condensation, and blood pressure regulation[15]. Neuroserpin also has roles that are independent or partly independent of its tPA inhibitory capacity. In this review, we aim to clarify the tPA-independent roles of neuroserpin in the CNS.

NEUROSERPIN'S STRUCTURE AND INHIBITORY ACTIVITY TOWARD PROTEASES

The serpin superfamily, which contains inhibitory and non-inhibitory members, is one of the earliest defined and largest protein superfamilies[16]. Serpins are

ubiquitously expressed in all branches of life, and are important regulators in many critical physiological processes, including coagulation, fibrinolysis, inflammation, metastasis and apoptosis[15]. The typical molecular structure of this family is very conserved and has been well defined, containing at least 30% amino acid sequence homology with the archetype protein, α1-antitrypsin. This protein is composed of eight or nine α-helices and three β-sheets, and characterized by an exposed peptide loop. This loop, named the reactive center loop, is composed of 20 residues and works as a pseudosubstrate for the target enzyme. The structural transformation between the reactive center loop and β-sheet is critical for the proteinase inhibition capacity[15, 17-19]. Neuroserpin is a fully functional inhibitor of trypsin-type proteinases, preferentially reacting with tPA and to a minor extent with the urokinase-type plasminogen activator (uPA) plasmin, but has no inhibitory activity towards thrombin[15, 20].

NEUROPROTECTIVE EFFECTS OF NEUROSERPIN DURING CEREBRAL ISCHEMIA

Ischemic stroke comprises approximately 88% of the total stroke, which is the leading cause of disability and the second largest contributor to mortality in the world[21]. It is often caused by an occlusion of a certain cerebral artery, and directly results in an absence of blood flow to this artery and the supplied brain tissue. Lack of oxygen and glucose could induce an energy metabolism disorder, which in turn triggers a collapse of ion gradients and an excessive release of excitotoxic neurotransmitters, such as glutamate and dopamine, ultimately leading to neuronal death and development of infarction[22].

One effective treatment for acute ischemic stroke is early thrombolytic therapy, and tPA is the only agent approved by the Food and Drug Administration for thrombolytic treatment. The therapeutic window is about 3 hours after the onset of stroke[23]. Meanwhile, tPA is capable of activating matrix metalloproteinase, degrading laminin, interacting with the N-methyl-d-aspartate receptor, and converting plasminogen to plasmin, which also contributes to blood-brain barrier degradation[22, 24-26]. These extravascular deleterious effects of tPA aggravate brain parenchyma injury when administrating tPA beyond its therapeutic window. This damaging side effect of tPA has also been shown by experiments carried out in the presence of tPA deficiency; these showed a significant reduction in ischemic lesion volume, an increase in neuronal survival, and a better long-term outcome[14]. Moreover, neuroserpin over-expressing mice and adjuvant treatment with neuroserpin both provided neuroprotective effects and increased the therapeutic window of tPA administration[12, 25, 27-29]. Based on these findings, it seems that the neuroprotective effect of neuroserpin following cerebral ischemia is associated with tPA, by balancing the proteolytic activity of tPA, or by regulating tPA mediated seizure spreading[13]. However, tPA has also been shown to rescue neurons from apoptosis induced by serum deprivation, and the elevation of tPA expression and activity induced ischemic tolerance and promoted neuronal survival in the murine hippocampus[30-31]. In these models, tPA worked as a neuroprotective agent like neuroserpin[32]. Additionally, a recent study showed that neuroserpin provides prominent neuroprotective effects in both wild-type and tPA-deficient mice during ischemic-hypoxic injury[33]. These findings suggest the existence of a tPA-independent neuroprotective role of neuroserpin during cerebral ischemia.

In fact, tPA is just one of the substrates of neuroserpin, which also reacts with uPA and plasmin[20]. A previous study showed that uPA was upregulated following ischemia, and that this could be inhibited by amiloride providing a better outcome[34]. The role of plasmin in the CNS is still unclear. A prior study indicated that plasmin was capable of activating protease-activated receptor-1 (PAR-1), and the activation of PAR-1 increased infarct volume by approximately 68% compared with PAR-1-null mice[35]. Neuroserpin had already been showed to abrogate the excitotoxic neuronal death induced by plasmin and kainic acid[33].

Methionine residues constitute an important antioxidant defense mechanism. There are 20 methionine residues in neuroserpin, and these contribute to the oxidative tolerance produced by this serpin protein. This molecular structural character of neuroserpin explains a part of its neuroprotective effects against oxidative stress[36].

It was reported that neuroserpin possessed anti-inflammatory activity in systemic arteries, modifying Th cell responses and reducing plaque growth[37]. Since systemic inflammatory stimuli are detrimental to stroke outcomes, we assume that the anti-inflammatory activity of neuroserpin also plays a neuroprotective role in cerebral ischemic conditions.

ROLES OF NEUROSERPIN IN TUMORIGENESIS

The human neuroserpin gene is localized to chromosome 3q26[38]. Despite its predominant expression in neurons, neuroserpin mRNA and/or protein has also been detected in pancreas, heart, kidney, testis, placenta, liver, pituitary and adrenal glands[20]. The role of neuroserpin in these tissues is still unclear, and recent research has shown that neuroserpin participates in tumorigenesis and tumor migration, including in brain tumor tissue[39-41].

The genomic locus of the programmed cell death gene PDCD10 is close to that of SERPINI1 (the gene encoding neuroserpin) and the two genes are oriented in a head-to-head configuration. SERPINI1-PDCD10 had

been found to be regulated by the oncogenic transcription factor c-Myc, and is possibly involved in CNS diseases such as brain tumors[42]. Moreover, there is evidence showing that the neuroserpin gene is downregulated in the brain tumor tissues and even absent in two brain cancer cell lines: U-87 MG and H4. These findings suggest that the neuroserpin gene is a cancer-associated gene, and that neuroserpin functions as a tissue-specific tumor-suppressor gene in the brain[39].

However, this tissue-specific tumor-suppressive character is not only observed with neuroserpin, but also with some other members of the serpin family, such as pancpin and maspin[43]. Mapsin was downregulated in breast cancer, like neuroserpin, while the transgenic high-expression of mapsin resulted in a reduction of tumor progression[15, 43]. Based on this, we predicted that neuroserpin may represent a new target for cancer therapy. The currently poor understanding of how neuroserpin produces its tumor-suppressive effect needs to be addressed. Contrarily, high neuroserpin gene expression was detected in high-grade prostate cancer and hepatocellular carcinoma, where it was associated with a poorer outcome[40-41]. This result suggests a need for suspicion towards the potential use of neuroserpin as a chemotherapeutic agent.

tPA converts inactive plasminogen into active plasmin, which is capable of degrading most of the extracellular matrix, and tPA itself degrades laminin and activates matrix metalloproteinase contributing to the degradation of extracellular matrix[22]. Sequentially, the breakdown of the extracellular matrix facilitates the invasion of cancer cells and enables tumor migration[44]. The absence of neuroserpin, a crucial inhibitor of tPA in the CNS, may promote brain tumorigenesis. The tPA-inhibitory function of neuroserpin is necessary in this tumor-suppressive process.

However, neuroserpin is not only a protease inhibitor: its expression has also been detected in pituitary and adrenal glands, suggesting a possible role for neuroserpin in the endocrine system. Both the AtT-20 cell line and PC12 cells, two endocrine cell lines, responded to altered neuroserpin expression levels by extending neurite-like outgrowths. Interestingly, compared with the parent and neuroserpin-overexpressing cell lines, there was only a small activation of tPA expression and no accumulation of tPA-neuroserpin complex was observed[45-46]. These results suggest an effect of neuroserpin in mediating neurite outgrowth, and that this process is tPA independent.

Lee *et al* [47], using PC12 cells treated with nerve growth factor, showed that the high expression of neuroserpin induced an increased level of cell-cell adhesion and higher N-cadherin expression. A similar outcome was detected in two mutant cell lines. One mutation was in the reactive site of neuroserpin, blocking recognition by tPA; the other mutation was at the hinge P_{14} serine residue, preventing reactive center loop insertion into β-sheet A, which resulted in a noninhibitory neuroserpin. This finding implied that neuroserpin could mediate cell-cell adhesion and alter the expression of N-cadherin, and this function was independent of its enzyme inhibitory activity.

These tPA-independent capabilities of neuroserpin, neurite outgrowth mediation and cell adhesion encouragement possibly result in the promotion of cancer cell metastasis and enhancement of the connection between tumor cells and local normal cells, promoting a secondary lesion. Although the innate protease inhibitory activity of neuroserpin provides us with a potential target for brain tumor treatment, the multiple tPA-independent roles of neuroserpin may lead an opposite result during tumorigenesis.

EMOTIONAL AND BEHAVIORAL REGULATION BY NEUROSERPIN

Neuroserpin and tPA participate in multiple mental processes and some motor neuron diseases[8]. These processes all require neuronal plasticity, but the mechanisms underlying their effects remain undefined. Madani *et al* [48] generated viable and healthy neuroserpin-deficient mice. Explorative behavior analysis revealed that these mice had a selective reduction of locomotor activity in a new environment, an anxiety-like response in the O-maze, and a neophobic response to novel objects. Surprisingly, zymographic analysis of brain extracts revealed unchanged tPA activity in neuroserpin-deficient mice[48]. This finding suggested that neuroserpin was a novel regulator of behavior and emotion, and that this role of neuroserpin was tPA independent. It also indicated that other inhibitors, such as PAI-1, which was found to form complex with tPA in demyelinating multiple sclerosis lesions[49], contributed to the regulation of tPA activity in the brain and compensated for the defect of neuroserpin activity.

As mentioned above, noninhibitory neuroserpin is also capable of mediating N-cadherin expression[47]. Through the Rho-family GTPases, N-cadherin plays a key role in synapse formation, controlling dendritic spine maturation, and participating in long-term potentiation[50]. Moreover, a higher level of neuroserpin expression can alter dendritic spine shape and induce an increase in the density of dendritic protrusions. This suggests a regulatory role of neuroserpin in neuronal structural plasticity[51]. Additionally, neuroserpin has a partly tPA-independent activity in promoting neurite outgrowth, while the extension and remodeling of neurites also play critical roles in neuronal development and plasticity[45-46]. Because neuronal plasticity is an essential composition for emotional and behavioral processes, the roles of neuroserpin in modulating N-cadherin expression, neuronal structural plasticity and neurite outgrowth reveal novel effects of neuroserpin in mediating emotion and behavior.

ROLE OF NEUROSERPIN IN FAMILIAL ENCEPHALOPATHY WITH NEUROSERPIN INCLUSION BODIES (FENIB)

Five point mutations of the neuroserpin gene, Ser49Pro, Ser52Arg, His338Arg, Gly392Glu and Gly392Arg, have been shown to induce deposition of ordered neuroserpin polymers within the neuronal endoplasmic reticulum (ER). This accumulation of polymers results in an autosomal-dominant dementia, called FENIB[15, 17, 20, 52]. FENIB patients show clinical features such as cognitive dementia, tremor, seizures and progressive myoclonus. A very clear genotype-phenotype correspondence has been revealed in this disease, which can be explained by the rate of polymerization[53]. For example, compared with Ser49Pro, patients with the Ser52Arg mutation exhibit more rapid polymerization, a greater number of inclusion bodies, an earlier age of onset and more serious clinical symptoms.

Although the lack of tPA-inhibitory activity of mutant neuroserpin, resulting in an imbalance of these two important proteins in the CNS, underlies the etiology of FENIB, the overload of ER stress seems to be more harmful. Normally, both active neuroserpin and neuroserpin-tPA complexes are recognized and internalized by membrane receptors, typically the LDL receptor-related protein, and degraded predominantly by ER-associated degradation (ERAD) and autophagy[54-55]. However, mutant neuroserpin significantly decelerates secretion and resists ERAD, resulting in a massive collection of neuroserpin polymers within the ER, dramatically increasing ER stress[15, 17, 20]. The overload of ER stress initiates cell death and leads to severe consequences. This ER overload damage has also been identified in liver cirrhosis caused by mutant α1-antitrypsin, in which the sequestration of mutant α1-antitrypsin from inclusion bodies is a cell-protective mechanism to maintain ER function[56]. Furthermore, the cellular toxicity of mutant neuroserpin was confirmed by a fly FENIB model and a rat FENIB model[57-58]. In these two models the extent of neuroserpin polymer accumulation was directly linked to the severity of locomotor deficits and a selective dysfunction or loss of a specific neuronal population, respectively. A recent study showed that neuroserpin polymers were capable of activating the calcium-dependent nuclear factor-κB pathway[59]. Because chronic activation of nuclear factor-κB could induce cell death, the regulation of nuclear factor-κB by neuroserpin polymers contributes to neuroserpin-associated cellular toxicity. This finding reveals a novel mechanism used by neuroserpin to achieve motor and cognitive regulation, and suggests a new strategy for the treatment of FENIB.

CONCLUSION

Neuroserpin is an important protein in the CNS. Through its interaction with its main substrate, tPA, to maintain a proteolytic balance, neuroserpin plays essential roles in both physiological and pathological processes. Considerable progress in the understanding of neuroserpin has revealed its tPA-independent roles. Importantly, neuroserpin is not only a tPA inhibitor, it also reacts with uPA and plasmin, and these interactions underlie neuroserpin's protective effects during cerebral ischemia. A better understanding of the roles of neuroserpin in the inflammatory system will widen its use in the treatment of adult stroke as well as neonatal hypoxic-ischemic encephalopathy. Additionally, neuroserpin is a tPA-independent mediator of neurite-like outgrowth, cell-cell adhesion, and N-cadherin and NF-κB expression. The tPA-independent regulatory effect of neuroserpin participates in tumorigenesis, as well as emotional and cognitive processes. Further characterization of the multiple roles of neuroserpin and its polymers will be helpful for developing new strategies to treat the associated diseases.

Author contributions: Jiao Ma wrote this minireview. Yu Tong validated the final version of this manuscript. Meng Mao and Dan Yu participated in providing funding.

Conflicts of interest: None declared.

Funding: This work was supported by the National Natural Science Foundation of China, No. 30700908, 30772343 and 30973215.

Acknowledgments: We are grateful to all the members of the Program for Changjiang Scholars and Innovative Research Team in University, No. IRT0935, and the colleagues of the Laboratory of Early Development and Injuries, West China Institutes for Woman and Children's Health, West China Second University Hospital, Sichuan University, China for their helpful discussions.

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(Edited by Yang N, Qiu ZH/Yang Y/Song LP)