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EDITORIAL HIGHLIGHT

A role for neuroserpin in neuron morphological development

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Read the full article '*The serine protease inhibitor neuroserpin regulates the growth and maturation of hippocampal neurons through a non-inhibitory mechanism*' on page 561.

Serpin, or serine protease inhibitor, represents the largest family of protease inhibitors that are found in almost all forms of life, including viruses, prokaryotes and eukaryotes. Serpin suppresses its targets by forming stable, covalent complexes with protease molecules (Law *et al.* 2006). One of the best known serpin family members, neuroserpin, is expressed mainly in neurons in both the central and peripheral nervous systems, starting from the late stage of neural development and remaining throughout adulthood. Neuroserpin can be detected in most areas of the neuron, including dendrites and axons, and is enriched at the axonal terminals (Borges *et al.* 2010). Following synthesis and processing in the endoplasmic reticulum, neuroserpin is supposed to be delivered to axons and dendrites. Although the exact site of secretion remains unclear, neuroserpin may be released into the extracellular environment at synaptic sites, possibly from the pre-synaptic compartment.

Neuroserpin is implicated in many neural-pathological conditions such as ischemic neuronal death (Wu *et al.* 2010), β-amyloid turnover in Alzheimer's disease (Fabbro *et al.* 2011), epilepsy (Hagen *et al.* 2011) and schizophrenia (Brennand *et al.* 2011), and has a neuroprotective function. The most striking pathological consequence is caused by neuroserpin polymerization. Mutations in neuroserpin lead to the formation of tangles of ordered polymers of neuroserpin in the endoplasmic reticulum, causing a form of dementia known as familial encephalopathy with neuroserpin inclusion bodies (Miranda *et al.* 2008).

This disease is characterized by eosinophilic neuronal inclusions of neuroserpin in the cerebral cortex and the substantia nigra (Roussel *et al.* 2011), with the most common symptoms being dementia and seizure. In the beginning, affected individuals may have difficulty in sustained attention and concentration. As the condition progresses, their personality will change, with judgment and memory becoming impaired. The role of neuroserpin in brain function is also demonstrated in transgenic animals. Altered emotional behavior such as neophobic response to novel objects has been observed in transgenic mice over-expressing neuroserpin (Madani *et al.* 2003). These observations indicate an important role for neuroserpin in brain development and neuronal function. However, whether and how neuroserpin affects neuronal growth and maturation remains less clear.

In this issue, Lee *et al.* (2012) for the first time provide evidence supporting a role for neuroserpin in the morphological maturation of neurons and neurite growth. The authors show that in cultured hippocampal neurons, protein levels of the cellular neuroserpin reached a peak at DIV 7, then decreased but remained at a relatively high level throughout the time of culture for 3 weeks in vitro. In contrast, the secreted extracellular neuroserpin was more age-dependent. A high level of neuroserpin was detected only at DIV7-14, showing only a minimal amount in the first week and after DIV14. As total cellular protein levels remained high until the third week, the reduced extracellular component suggests an age-dependent regulation in neuroserpin release or more efficient degradation. Incubation of cultured hippocampal neurons with recombinant neuroserpin induced a reduction in the diameter of dendrites and in the size of the soma and the axonal growth cone. These changes might result from facilitated removal or reorganization of the lamellipodia structure distributed around the soma, the

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Abbreviations used: LRP1, low density lipoprotein receptor-related protein 1; RAP, receptor-associated protein; tPA, tissue plasminogen activator.

immature dendrites and the growth cone. Analysis of neurite development showed that neuroserpin treatment had led to an increase in axon branching and thus the total axon length. Different effects were observed in dendrite growth. Neuroserpin increased dendrite growth and branching at the early developmental stage, whereas simpler dendritic branching was observed in more mature neurons. A plausible scenario is that neuroserpin first promotes random growth of new dendrites before helping in the pruning of non-essential branches.

Because no changes were found in synapse density along the dendrites, neuroserpin may not have a major impact on synaptogenesis. Nevertheless, the authors found enlarged synaptic puncta of both pre-synaptic terminals and post-synaptic sfs density-95 (PSD-95)-positive domains. As earlier work from the same group demonstrated a neuroserpin-induced increase in dendritic spines (Borges *et al.* 2010), the lack of change in synapse density indicates that neuroserpin is not sufficient to promote the formation of functional synapses.

The report shows that the protease inhibitory activity of neuroserpin is not required for the observed effects. Neuroserpin has been shown to inhibit multiple serine proteases including tissue plasminogen activator (tPA), urokinase-type plasminogen activator, trypsin, plasmin and thrombin, of which tPA is considered the primary substrate. Given that tPA is involved in spine growth and synaptic plasticity (Horii-Hayashi *et al.* 2011), the effect of neuroserpin on neuron morphogenesis is likely mediated via tPA inhibition. Surprisingly, incubation of neurons with two non-inhibitory forms of neuroserpin induced the same effects as those with wildtype neuroserpin. Furthermore, utilization of a different, but closely-related serpin that also inhibits tPA produced no effect on neuronal growth, indicating that the observed effect is tPA-independent and serpin subtype (neuroserpin) specific.

The molecular mechanisms and signaling cascades underlying the effect of neuroserpin remain unclear. The authors attempted to explore the involvement of low density lipoprotein receptor-related protein 1 (LRP1), a known binding partner of neuroserpin. However, the results are complicated by the fact that the LRP1 receptor antagonist receptor-associated protein (RAP) itself also induced morphological changes in neurons. Neuroserpin plus RAP causes further morphological changes over those caused by RAP alone, suggesting that the effects of neurosepin may be mediated, at least in part, by LRP1-independent pathways. Indeed, despite its major role in the inhibition of serine proteases, serpin has been shown to suppress other proteases, or participate in more diverse non-inhibitory effects (Olson and Gettins 2011). In light of this, additional work is needed to elucidate the molecular details of neuroserpin-dependent signaling, as well as the role of neuroserpin in neuronal functions such as synaptic transmission and plasticity.

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