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Stroke Literature Synopses: Basic Science (2015/February)

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After stroke onset, neurons undergo several deleterious signaling cascades. Apoptosis is one of the major mechanisms that lead to neuronal death after stroke or other neurodegenerative diseases. However, apoptotic mechanisms in neurons still remain to be fully elucidated. Three recent studies suggest novel mechanisms for neuronal apoptosis under pathological conditions.

Baik et al. (Pin1 promotes neuronal death in stroke by stabilizing Notch intracellular domain. *Annals of Neurology*. 2015;doi:10.1002/ana.24347) examined the mechanisms as to how the peptidyl-prolyl cis/trans isomerase Pin1 contributes to neuronal apoptosis. Pin1 acts as a molecular switch in cells by activating or deactivating specific kinases/phosphatases. Pin1 may play important roles in neuronal apoptosis under some conditions including oxidative stress or glutamate toxicity, but how Pin1 behaves under ischemic stroke conditions was yet unknown. First, the authors investigated the role of Pin1 in Notch1 activation because Notch signaling is activated under ischemic conditions and Pin1 interacts with many signaling proteins including one related to Notch signaling. Molecular and cellular experiments demonstrated that Pin1 bound to and stabilized the Notch Intracellular Domain, leading to Notch1 activation. In cell culture systems, ischemic conditions increased Pin1 expression that could potentiate cell death via accumulating the Notch Intracellular Domain. Furthermore, the authors used an in vivo mouse stroke model by middle cerebral artery occlusion to demonstrate that Pin1 knockout mice exhibited lower expression level of the Notch Intracellular Domain. In addition, in the mouse model of stroke, the Pin1 inhibitor juglone was effective in that it reduced neurological deficits and infarct size.

Epigenetic modification mechanisms are also involved in neuronal apoptosis after stress.

Peng et al. (HDAC2 selectively regulates FOXO3a-mediated gene transcription during oxidative stress-induced neuronal cell death. *The Journal of Neuroscience*.

2015;35:1250–1259) examined how forkhead box O3a (FOXO3a) is involved in neuronal death. FOXO3a is a transcription factor, and is known to be involved in several physiological and pathological responses including apoptosis. In this study, the authors tested the hypothesis that histone deacetylases (HDACs), which are enzymes that modulate histone acetylation, would mediate oxidative stress-induced neuronal apoptosis in a FOXO3a-dependent manner. First, using the tandem affinity purification assay and co-immunoprecipitation assay, the authors showed that both HDAC1 and HDAC2 interact with

FOXO3a. Under ectopic expression conditions in 293T cells, both HDAC1 and HDAC2 formed a complex with FOXO3a. But HDAC1-FOXO3a and HDAC2-FOXO3a complexes may play different roles in cell survival/death because in neuronal cultures, HDAC2 knockdown, but not HDAC1 knockdown, protected neurons from H₂O₂-induced apoptosis. For the underlying mechanisms, the authors demonstrated that FOXO3a recruited HDAC2 to the p21 promoter, which blocks p21 expression. Phosphorylation of HDAC2 at Ser 394 was shown to be crucial for the binding of HDAC2 to FOXO3a. Importantly, HDAC2 inhibition promoted p21 expression, which protected neurons from oxidative stress-induced apoptosis both in *in vitro* neuron cultures and *in vivo* mouse stroke model by middle cerebral artery occlusion.

microRNAs (miRNAs) are important regulators for cellular homeostasis, and changes in miRNA expression/activity may cause cell death/damage. In terms of neuronal function, miRNAs are known to regulate synaptic signaling, especially in postsynaptic responsiveness during synaptic transmission. **Verma et al. (A neuroprotective role for microRNA miR-1000 mediated by limiting glutamate toxicity. *Nature Neuroscience*. 2015;doi: 10.1038/nn.3935)** used *Drosophila* models to examine the roles of miRNAs in presynaptic regulation, focusing on miR-1000. Genetic ablation of *miR-1000* increased the level of vesicular glutamate transporter (VGluT), which loads glutamate into synaptic vesicles. Concomitantly, the mutant showed elevated apoptosis in the brain due to the excessive glutamate release from presynapse. *miR-1000* is not found in mammals. But the seed-similar miRNA *miR-137* is conserved, and the authors then examined whether miR-137 regulates VGluT in mammalian neurons. When miR-137 was depleted in mouse cortical neuron cultures, an increase in VGluT mRNA level was observed, accompanied with more caspase3-positive cells. Furthermore, when miR-137 was overexpressed in the dentate gyrus region of the hippocampus, elevated VGluT protein levels were observed, indicating that the VGluT regulation mechanisms by miR-1000 in presynapses are conserved in mammals.

Beyond caspases *per se*, neuronal apoptosis may involve a much broader network of regulatory signals. A better understanding of these networks may allow us to pursue more rigorous ways to block neuronal apoptosis for therapeutic gain.