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Neurogenesis and Apoptotic Cell Death

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As recently as a decade ago, it seemed inconceivable to most neuroscientists that new neurons could be born routinely in the adult mammalian brain (neurogenesis) and that neurons and other brain cells die by committing a form of cell suicide (apoptosis). Both fields have evolved into large research enterprises that continue to revolutionize how we think about limiting cell injury and cell death in the adult brain and spinal cord. Moreover, both have afforded new possibilities that one day may help to restore normal function to the injured nervous system. In fact, this past year's Nobel prize in Physiology and Medicine was awarded to Robert J. Horvitz of the Massachusetts Institute of Technology for his pioneering experiments elucidating complex pathways promoting and blocking programmed cell death in the nematode *C elegans*. Horvitz's elegant discoveries showed how genes and proteins expressed within evolutionarily ancient and biologically less complex organisms can profoundly influence our understanding of cell death in the mammalian nervous system, with equally important implications for the developing brain and for brain cancer. This review examines recent advances underscoring the importance of neurogenesis and neuronal cell death to the adult brain after stroke.

Neurogenesis

Replenishing brain cells from endogenous and exogenous sources is one of the most exciting frontiers in stroke research.[1,2] In normal adult brain, neuroblasts from the subventricular zone (SVZ) migrate via the rostral migratory stream toward the olfactory bulb, whereas neuroblasts from the subgranular layer mostly supply the hippocampus. After transient global ischemia, precursor cells within the SVZ and subgranular layer proliferate and differentiate into neurons (neurogenesis) and other brain cells.[3] Although neurogenesis is modest after injury, it can be significantly enhanced by treatment with growth factors.[4] Remarkably, Nakatomi and colleagues[5] found that administering epidermal growth factor combined with fibroblast growth factor 2 replenished up to 50% of CA1 neurons in the damaged hippocampus, a month after 6 minutes of global ischemia in rats. Furthermore, electrophysiological recordings from recently born hippocampal neurons suggested that they made appropriate neuronal connections and exhibited long-term potentiation. In addition, neurological performance in treated rats improved significantly on a Morris water maze, a crude test of hippocampal function. These unprecedented findings imply that growth factors and substances that can significantly stimulate the birth of new neurons may already be within our molecular armamentarium.

Progenitor cells with a more restricted fate than those in the SVZ or subgranular layer may also reside within cerebral cortex and provide a local source of new neurons as demonstrated in a rat model of cortical apoptosis triggered by focal photochemical injury.[6] Thus, it remains conceivable that multiple brain areas function as repositories or reservoirs of neural progenitor cells. In the most hopeful scenario, these cells can proliferate, differentiate, and integrate into injured and normal tissue to achieve a meaningful neurological recovery.

An important question addressed by Arvidsson et al[7] and by Parent et al[8] this past year is whether brain areas not normally targeted by endogenous precursors can be repopulated by newly born cells. The evidence suggests that they can. Two weeks after transient focal ischemia in rats, chains of migrating neuroblasts divert from the rostral migratory stream and redirect toward the damaged striatum. Newly born cells express neuronal markers such as dopamine and adenosine 3′,5′ -monophosphate–regulated phosphoprotein of 32 kDa (DARPP-32), as in other projecting striatal neurons. The precise signaling and environmental cues directing cell migration remain unknown.

Replenishment of brain cells from exogenous sources (transplantation) provides a complementary approach to endogenous cell replacement that is equally exciting. In a rat focal ischemia model, implanted MHP36 murine stem cells differentiated into neurons and significantly improved neurological recovery at 3 months.[9] In another provocative study, intravenously injected bone marrow stromal cells migrated into stroke-damaged brain and stimulated the growth of new blood vessels.[10] Implanted stem or stromal cells may also stimulate endogenous neurogenesis by producing growth factors. Injected bone marrow cells synthesize brain-derived neurotrophic factor and nerve growth factor, reduce apoptotic cell death in penumbra, enhance endogenous neurogenesis, and improve neurological recovery after focal ischemia in rats.[11] Although the signaling pathways that promote neurogenesis remain to be fully dissected, nitric oxide and cGMP have been recently implicated.[12]

In developing brain, the extracellular matrix provides both stimulatory and inhibitory signals that modulate neuronal migration, neurite outgrowth, and axonal extension. After injury to adult brain, reexpression of inhibitory molecules such as chondroitin sulfate proteoglycans may prevent damaged tissue from regenerating. Chondroitinase degrades these inhibitory substrates, and when infused in a rat model of spinal cord injury, enhanced the regeneration of axons in dorsal column tracts and improved locomotor recovery.[13] Similar approaches may be feasible in stroke. In mammalian brain, the myelin inhibitory protein NOGO together with myelin-associated glycoprotein and oligodendrocyte glycoprotein plays a critical role in modulating axonal extension.[14] Targeted antibodies against NOGO neutralized this inhibitory system, promoted neuronal regrowth and axonal reconnections, and improved functional recovery after focal cerebral ischemia in rats.[15] Ultimately, strategies that replace damaged neurons; enhance endogenous neurogenesis; and suppress inhibitory systems will best be approached by using combined therapies. However, that will require a greater understanding of both temporal and spatial events developing in series and parallel within the recovering nervous system.

Apoptotic Cell Death

Although published evidence for the existence of apoptotic cell death in the human brain is still limited, results from experiments in other mammalian species strongly suggest that neurons and glia may die by a mechanism resembling apoptosis. Over the past 5 years, the data generated in vivo in rats and mice and in cultured cells indicate that executioner enzymes, the caspases, are expressed in neurons and become activated during and after ischemic stress and in neurodegenerative diseases. Active caspases kill cells by cleaving critical cell repair and homeostatic proteins as well as cytoskeletal proteins. Neurons from mice genetically deficient in caspase 3 are relatively resistant to cell death caused by oxygen-glucose deprivation in vitro and to mild brain ischemia after middle cerebral artery occlusion.[16] Pharmacological evidence showing that caspase inhibition suppresses the size of ischemic injury is consistent with these results. Because caspases are constitutively expressed at high levels by immature brain, caspase-mediated cell death may be especially relevant to neonatal ischemia. In fact, treatment of the neonate with a novel selective nonpeptide inhibitor of caspase 3 reduced injury and blocked caspase 3 activation in a recent brain ischemia study.[17] (Unfortunately, the tested compound poorly penetrates the blood–brain barrier, so that it is impractical to test in humans.) Caspase-dependent cell death is especially prominent in tissues sustaining milder injury and for good reason.[18] ATP levels in this territory, but not in the core, are sufficient to organize the apoptosomal complex and thereby activate downstream executioner caspases.

Despite encouraging preclinical results, it needs to be emphasized that necrosis and not apoptosis is the predominant cell death mechanism, although many dying cells exhibit features of both. Apoptosis and necrosis are triggered by ligation of death receptors such as by tumor necrosis factor-α engagement.[19] Based on recent

work, we now also know that apoptosis can be suppressed by phosphorylating enzymes or kinases such as Akt (eg, block caspase 9) or modulated by MAP kinase pathways to either increase or reduce apoptosis. Heat shock proteins regulate the death pathway and cell fate by binding key proteins in the apoptosis cascade. Not surprisingly then, mechanisms of cell death in brain cells are highly redundant, tightly regulated, and very complex.

The year 2002 also witnessed the emergence of novel apoptotic cell death pathways that are caspase independent. One of these pathways involves the release of apoptosis-inducing factor (AIF). AIF, discovered in 1999,[20] is a 67-kDa flavoprotein stored within the same mitochondrial compartment as cytochrome c (a promoter of caspase-dependent cell death on release). The role of AIF in mitochondrial function is unknown.[21] DNA damage and oxidative or excitotoxic stress release AIF. The nuclear enzyme poly(ADP)-ribose polymerase was recently implicated as a trigger.[22] On its relocation from the mitochondrial intermembranous space, AIF translocates to the nucleus where it binds to DNA, promotes chromatin condensation, and kills cells by a complex series of events exhibiting an apoptosis phenotype. Cell death by AIF seems resistant to treatment with pan-caspase inhibitors but is blocked partially by Bcl-2.[22] In brain ischemia, AIF was reportedly released from mitochondria in preliminary reports. Hence, there is a compelling need for more detailed information about AIF and caspase-independent cell death and better tools to manipulate them.[23]

This year Cao and colleagues[24] developed and tested a Bcl-xL fusion protein that promotes transport of Bcl-xL across the blood-brain barrier and into neurons, enabled by a protein transduction sequence derived from the human immunodeficiency TAT protein.[25] Bcl-2 and its related family member Bcl-xL are among the most powerful death-suppressing proteins and inhibit both caspase-dependent and caspase-independent cell death. After intraperitoneal injection, protein transduction was evident within neurons, and importantly, ischemic injury was significantly attenuated after focal cerebral ischemia in treated mice.[24] Such findings are notable because they reaffirm the death-suppressing function of Bcl-xL in ischemic injury and establish that this technology can efficiently transduce a large molecular weight and powerful neuroprotectant into ischemic brain.

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Footnotes

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