Cell cycle regulation, neurogenesis, and depression

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The mammalian brain was originally thought to birth new neurons only during development. A modern paradigm shift began in the 1960s as experimental evidence of neurogenesis in adult brain and its functional implications began to emerge. Neural stem cells have now been identified to undergo mitosis, proliferate, and differentiate into neurons, astroglia, or oligodendroglia within specialized neurogenic zones that interdigitate especially regions of the corticolimbic brain (e.g., hippocampus, olfactory bulb, amygdala, and cerebral cortices) (1). Multiple chemical and behavioral factors trigger or suppress neurogenic processes, including trophic factors (e.g., growth factors), neurotransmitters [e.g., serotonin (5-HT)], hormones (e.g., glucocorticoids, estrogens), and exposure to stress, physical activity, learning situations, neurotoxins, and other forms of brain damage (1). Thus, neurogenesis is thought to underlie memory processes and may explain several neurological and psychiatric disorders, although contradictory evidence, controversy, and unanswered questions remain.

The discovery of adult neurogenesis has spawned investigations of its molecular mechanisms and role in brain disorders, both those considered neurological (e.g., Alzheimer's) and psychiatric (e.g., depression). In a recent issue of PNAS, Pechnick *et al.* (2) demonstrate that antidepressant treatment down-regulates expression of the cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1}$ (p21), a suppressor of cell cycle traverse and, thus, proliferation. This down-regulation of p21 induced by *in vivo* imipramine administration tracks with both increased hippocampal neurogenesis and increased antidepressant-like behavioral effects. The basal level of proliferation of hippocampal neuroblasts was also elevated in mice that lack the p21 protein. These data suggest a mechanistic link between neurogenesis and the actions of antidepressant treatment.

An appreciation of the importance of the Pechnick *et al.* research (2) necessitates connecting the hypothetical dots between neurogenesis, cell cycle, and antidepressant effects, which this group has accomplished. As for other somatic cells, neuroblast proliferation is regulated during development by a balance of inhibitory and excitatory signals (3). Cyclin-dependent kinases drive the cell cycle through its phases via phosphorylation of downstream proteins. Of the four phases of the cell cycle, G_1 is a dynamic stage marked by high rates of biosynthesis and responsiveness to extracellular regulatory signals required for the cell to progress toward mitosis. Control of the G_1 phase is an essential gatekeeper in the rate of cell cycle progression, and is negatively regulated by two main families of CDK inhibitors: the Ink4/ARF family and Cip/Kip-type family. The CDK inhibitor p21 maintains cell quiescence, blocking progression into S-phase (3). Building on observations that p21 expression in brain is sustained postnatally, Pechnick *et al.* (2) conducted an elegant series of experiments that linked the powers of confocal microscopy, fluorescent-activated cell sorting, and immunocytochemistry to discover that p21 expression occurred exclusively in neuroblasts, not mature, hippocampal neurons; in null p21 mice (−/−), the rate and extent of hippocampal neurogenesis increased significantly. These data support the hypothesis that proliferation of adult neurons is restrained by CDK inhibitors and that a loss of p21 function results in the release of progenitors from cell cycle block. Importantly, these studies provide evidence that cell cycle regulatory processes observed to occur during neuronal development are operable in adult neurons as well as adult glia (1), and serve as a point of manipulation of neurogenesis for the future.

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Beyond the Monoamine Hypothesis: Depression and Neurogenesis

Pechnick *et al.* (2) importantly demonstrate that the mechanisms underlying the efficacy of antidepressants, and perhaps the etiology of some depressive disorders, may include actions at the level of the cell cycle. Depression is a debilitating disorder of mood and cognition for which multiple diagnostic categories are described (e.g., major depression, bipolar disorder). The monoaminergic hypothesis that depression results from a deficiency of 5-HT and norepinephrine (NE) function has dominated the landscape of the field for >30 years, supported largely by the effectiveness of antidepressant therapies that prevent the reuptake [tricyclic antidepressants, selective 5-HT reuptake inhibitors (SSRIs)] or metabolism (monoamine oxidase inhibitors) of these transmitters. Inhibition of reuptake by all classes of antidepressants occurs within minutes; however, clinical improvement is seen only after 4–6 weeks, suggesting that a cascade of neural events triggered by enhanced synaptic levels of monoamines must ultimately engage neuroplastic adaptations that reset the functional imbalance in depression. A myriad of changes in expression/function of monoamine reuptake transporters and receptors have been observed, but have not adequately explained the time course of efficacy of chronic antidepressant treatment (4). The contemporary focus has shifted to consider downstream events to help define a common final pathway that explains the time delay in pharmacotherapy (4).

One candidate mechanism proposed as underlying the therapeutic efficacy of antidepressants is the induced growth of new neurons and restoration of normal function in adult corticolimbic brain. In keeping with this hypothesis, all major classes of antidepressants stimulate neurogenesis on a time course that tracks with emergence of their therapeutic effects (5), whereas suppression of hippocampal neurogenesis abolished the behavioral effects of antidepressants (6). A corollary hypothesis is that the etiology and progression of depression may involve neurodegeneration and impairments of neural webs and connectivity in limbic brain. The neurogenesis hypothesis of depression is attractive but

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highly controversial, as recently reviewed (7–9). Neurodegenerative processes in depression are supported by observations of decreased gray matter volume of hippocampus and frontal and temporal cortices (structural imaging) (10, 11) and neuronal and glial pathology in depressed patients (postmortem) (12). However, decrements in stem cell proliferation (13) or numbers of neurons in hippocampus (12) have not yet been observed in postmortem studies of depressed patients. Nor does impaired neurogenesis appear to be a precondition for depressive-like behaviors in animal models (14). Although the controversies about causal links between neurogenesis and brain disorders rage, neuroscientists cannot deny the excitement of adult neurogenesis, highlighting the need to further identify the functions of these new neurons and discover the regulatory processes of neurogenesis in the adult brain.

Antidepressant Actions and Cell Cycle Regulation

Pechnick *et al.* (2) addressed this last goal to study cell cycle regulation of neurogenesis in adult brain. Pechnick *et al.* postulated that hippocampal neurogenesis and the effects of the antidepressant imipramine may involve the cell cycle regulator p21. An up-regulation of hippocampal neurogenesis in the subgranular zone (SGZ) of the rat hippocampal dentate gyrus following 21 days of imipramine treatment was demonstrated by increased incorporation of bromodeoxyuridine (BrdU; labels new DNA in the S phase of the cell cycle) as well as increased expression of neuronal nuclear protein (NeuN; marker of neurons) and proliferating cell nuclear antigen (PCNA; marker of cellular proliferation). The imipramine regimen also resulted in the expected profile of behaviors (decreased immobility and increased escape behaviors) in a well described model of antidepressant activity (forced swim test) (15). A concomitant decrease in p21 mRNA and protein expression was seen in the SGZ. Thus, the neurogenic and behavioral effects of imipramine might be linked to the release of a p21-mediated restraint of the cell cycle in hippocampal neuroblasts.

The signaling cascade that underlies antidepressant-induced alterations of p21 is unknown. Imipramine inhibits the reuptake of 5-HT, but also inhibits NE reuptake, and has affinity at multiple monoamine receptors (16). G protein-coupled receptors are known to regulate cell cycle progression in multiple cell types (17) and activation of 5-HT and NE receptors and subsequent receptor-linked intracellular signaling pathways have been shown to regulate DNA synthesis, cell cycle progression and/or other mitogenic signals for cellular proliferation (18, 19). For example, exposure to selective 5-HT receptor agonists (i.e., 5-HT_{1A}, 5-HT_{2C}, 5-HT_4 receptors) are consistently reported to increase neurogenesis in the SVZ, other hippocampal regions and/or the olfactory bulb (20, 21). Thus, the indirect actions of imipramine to activate membrane-bound monoamine receptors may be able to trigger associated intracellular cascades with a final effect to regulate cell cycle, and ultimately cellular proliferation.

The biological secrets of the depressed brain are yielding to modern science. Clearly, the dance between life and death of neurons in the central nervous system is a tightly regulated process and specific aberrations in this balance may be an important causal factor in depressive disorders or underlie some aspects of the therapeutic response to antidepressants. The advances presented by Pechnick *et al.* (2) broaden the focus to urge further elucidation of cell cycle and its regulators in adult neurogenesis. Because cell cycle regulators, including p21, also control proliferation and survival of brain tumor stem cells, attempts to consider such regulators as "druggable" therapeutic targets require a much greater appreciation of their relative oncogenic and tumor suppressor roles in both normal and tumorigenic neural stem cells (22). Whether a causal link between neurogenesis and depression is ever eventually validated, further research is needed to refine the molecular mechanisms underlying neurogenesis in response to external and internal stimuli, and identify the place of other important factors in the immediate upstream and downstream cellular signaling web (other kinases, growth factors, cytokines) that controls adult neurogenesis.

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