Genetics of Childhood Disorders: XXXVI. Stem Cell Research, Part 1: New Neurons in the Adult Brain

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Until recently, it was assumed that neurogenesis, or the production of new neurons, occurs only during development and never in the adult organism. The famous neuroanatomist Cajal stated that "Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In adult centers, the nerve paths are something fixed and immutable: everything may die, nothing may be regenerated." This statement holds true for most of the regions of the adult brain. However, there are two adult brain areas in which neurogenesis is observed: the subventricular zone of the anterior lateral ventricles gives rise to cells that become neurons in the olfactory bulb, and the subgranular zone in the dentate gyrus generates new granule cell neurons in the hippocampus, a brain region that is important for learning and memory.

The initial studies that suggested that the adult brain could generate new neurons were largely ignored. In the 1960s Joseph Altman and coworkers published a series of papers reporting that some dividing cells in the adult brain survived and differentiated into cells with morphology similar to neurons. They used tritiated thymidine autoradiography to label the cells. Tritiated thymidine is incorporated into the DNA of dividing cells. They found that the highest density of labeling was in the subventricular zone and in the dentate gyrus of the hippocampus. It was known that the dentate gyrus of the hippocampus is essentially devoid of glia. Therefore, Altman attributed the labeling in this region to the uptake of thymidine by dentate granule cells. However, he could not prove that the adult-generated cells were neurons rather than glia, since no phenotypic markers were available that could be used in conjunction with thymidine autoradiography. The absence of specific markers for neurons and glia and continued skepticism surrounding the novel concept of adult neurogenesis limited further development of the research.

In the mid 1970s and the early 1980s, Michael Kaplan and his colleagues reexamined the initial observations using the electron microscope and added substantial confidence that neurogenesis could occur in the adult brain. Combining electron microscopy and tritiated thymidine labeling, they showed that

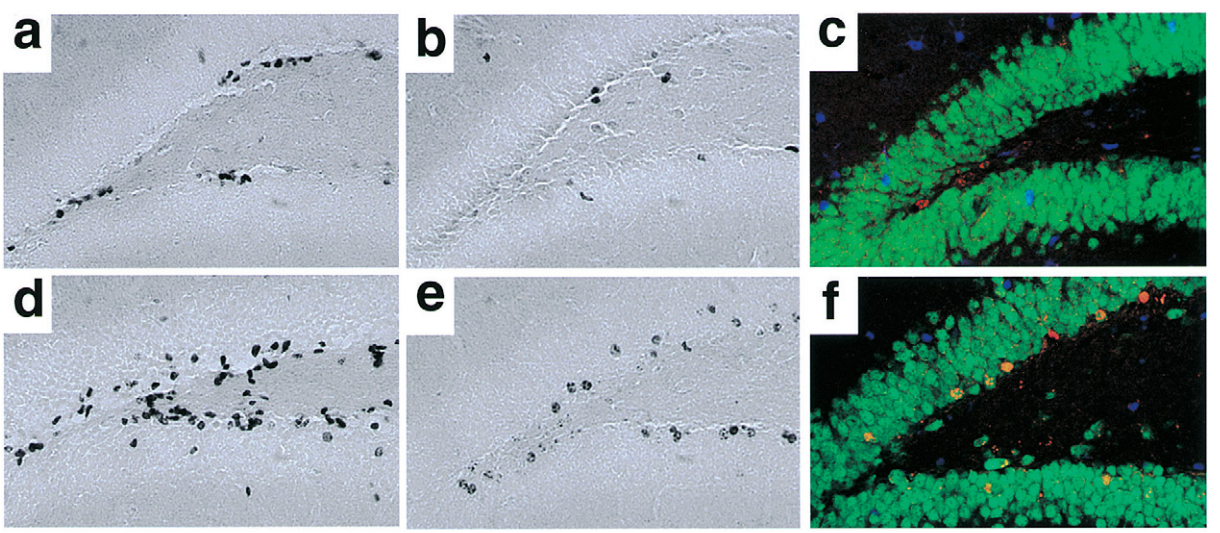

Fig. 1 Regulation of neurogenesis by exercise. Photomicrographs of BrdU-positive cells 1 day (a, d) and 4 weeks (b, c, e, f) after the last of a series of 12 BrdU injections. Mice that are housed with a running wheel (d, e) have more BrdU-labeled cells than sedentary mice (a–c). Running increases cell proliferation at 1 day after the last BrdU injection in runners (d) compared with controls (a). In addition, cell survival is enhanced 4 weeks after the last BrdU injection in runners (d, e) relative to controls (b, c). Confocal images (c, f) of sections were immunofluorescent triple-labeled for BrdU (red), NeuN indicating neuronal phenotype (green), and s100β selective for glial phenotype. BrdU-positive neurons are orange (red plus green).

labeled cells in the rat dentate gyrus have ultrastructural characteristics of neurons, such as dendrites and synapses. However, most researchers did not consider this to be evidence of significant neurogenesis in adult mammals. It was still not possible to prove that the new cells were neurons. In addition, the concept that there may be brain stem cells that could proliferate, migrate, and then differentiate into new neurons had not yet been introduced. It was therefore thought that mature neurons would have to replicate, an idea that most researchers found incredible. Furthermore, the possible relevance of the findings for humans was underestimated because there was no evidence of neurogenesis in primates.

In the mid 1980s, astonishing findings in adult canaries by Fernando Nottebohm and his student Steve Goldman stimulated this fledgling field. They discovered that neurogenesis occurs in brain areas that mediate song learning. Using a combination of tritiated thymidine with ultrastructural and electrophysiological techniques, they provided evidence that the new cells were neurons. In addition, they showed that there is a peak in the production of new neurons at the time of year birds acquire songs. Nottebohm and his coworkers also showed that neurogenesis in the hippocampal complex of adult chickadees is correlated with seed-storing behavior. They focused on the hippocampus because this structure in relatively larger in seed-storing than nonstoring birds and because it plays an important role in spatial learning. Chickadees store seeds in the fall and then retrieve them after days or weeks. In one study, the birds were captured at different times of the year, injected with tritiated thymidine, released, and recaptured 6 weeks later. It was found that there was significant seasonality in the number of hippocampal cells labeled with tritiated thymidine. Birds that had received the label in October had more labeled cells than chickadees that had received the label at other times during the year. Taken together, these results raised the possibility that new neurons play a functional role in the mature brain and led to a revived interest in possible neurogenesis in adult mammals.

Despite the observations of neurogenesis in the adult avian brain, confusion over the mechanism of cell genesis in the adult brain persisted. In the early 1990s, a series of experiments in the adult mouse by Bartlett in Australia; Reynolds, Weiss, and coworkers in Canada; and Temple in the United States, and in the adult rat by Ray, Gage, and colleagues, revealed that cells with stem cell properties could be isolated and expanded in culture. Under a variety of culture conditions with different factors, these isolated cells can be induced to proliferate and differentiate into glia or neurons. Specifically, fibroblast growth factor and to a lesser extent epidermal growth factor stimulate proliferation of progenitor cells in culture. Factors that have been found to be important for neuronal differentiation in cultured progenitor cells are retinoic acid and cyclic AMP. In addition, neurotrophins such as NGF, BDNF, and NT-3 have been found to influence neuronal differentiation and transmitter phenotype, whereas CNTF can regulate glial differentiation of precursor cells. These observations in vitro provided a mechanism for the neurogenesis in the adult brain in vivo. Mature committed neurons were not dividing. A population of immature stem-like cells exists in the brain. It is likely that the proliferation and differentiation of this population results in neurogenesis.

It is interesting that immature or stem cells that can divide and give rise to neurons in culture can be isolated from many areas of the adult brain and spinal cord, not just from the subventricular zone and hippocampus. Stem cells also have been isolated from areas that are non-neurogenic such as the septum, striatum, spinal cord, cerebral cortex, corpus callosum, optic nerve, and eye. In culture, these cells are multipotent and can give rise to neurons and glia. This suggests that the potential for neurogenesis exists throughout the nervous system but that the signals necessary for neurogenesis have been lost or that inhibitory mechanisms may prevent its occurrence. Understanding these processes may have important implications for repair and recovery from injury in the adult brain.

In the 1990s, research pertaining to neurogenesis in vivo made great conceptual and technical progress. Researchers Stanfield and Trice showed that tritiated thymidine–labeled cells in the dentate gyrus project axons to hippocampal area CA3, the target for mature granule cell neurons. In their experiment, they injected the retrograde tracer fluorogold into area CA3 of tritiated thymidine–treated animals. Subsequently, cells that were double-labeled for tritiated thymidine and fluorogold were observed. Another important step forward was the use of the thymidine analog 5-bromo-3′-deoxyuridine (BrdU), a traceable analog of uridine, which is incorporated into the genome of cells undergoing cell division. The advantage of BrdU over thymidine autoradiography is that the cells can be visualized by using immunocytochemistry. This method allows for a more accurate estimate of the number of new cells with stereological techniques. In addition, BrdU immunocytochemistry can be used in combination with now available specific markers for neurons, such as NeuN and calbindin, and for glia, such as s100β and glial fibrillary acidic protein (GFAP). Double-labeling for BrdU and NeuN can be used to demonstrate convincingly whether a newborn cell has become a neuron.

Kuhn and coworkers in the Gage laboratory were the first to use BrdU labeling in combination with neuronal and glial markers, demonstrating that neurogenesis occurs throughout the lifespan of the adult rodent. They also showed that there is a time course over which neurogenesis occurs. When BrdU-labeled cells were examined a few days after the last BrdU injection, the majority of the cells did not co-label for any of the mature neuronal or glial markers; this finding suggests that these are immature, proliferating cells. At 4 weeks after the last BrdU injection, about 60% of the BrdU-positive cells co-labeled with the neuronal marker NeuN.

Until the 1990s, studies that provided evidence for neurogenesis were considered irrelevant to the primate or human brain. It was assumed that neurogenesis has become restricted throughout evolution, as the brain became more intricate. Thus lizards and other reptiles can regenerate and replace neurons after being damaged, whereas in the complex human brain, the addition of new neurons could conceivably disturb the intricate wiring of neuronal connections. A few years ago, however, Gould, McEwen, Fuchs, and colleagues provided evidence for neurogenesis in the hippocampus of the primate-like tree shrew and also in the marmoset monkey. Studies using rhesus monkeys, which are evolutionarily closer to humans than marmosets, by these investigators as well as by Rakic and Kornack confirmed that neurogenesis occurs in adult nonhuman primates.

At about the same time, the possible occurrence of neurogenesis in humans was studied. Administering BrdU and then examining cell proliferation in tumor biopsies is occasionally used to monitor tumor progression in patients with cancer. Because BrdU is a small soluble molecule, it is distributed throughout the body, including the brain, and thus can be a marker for cell division and neurogenesis in humans. In 1998, Eriksson, Gage, and coworkers reported that five cancer patients who had received BrdU between 15 days and more than 2 years earlier showed neurogenesis as revealed by co-labeling of BrdU with markers of mature neurons in the dentate gyrus. These studies clearly demonstrate that neurogenesis, at least in the dentate gyrus, is a process that persists throughout life in mammalian species, including humans.

Although the genesis of new neurons in the dentate gyrus of the hippocampus of adult mammals is now a generally accepted phenomenon, the functional role of the new neurons remains unclear. A variety of environmental, behavioral, genetic, neuroendocrine, and neurochemical factors can influence the proliferation and survival of newborn cells. For example, researchers in the Gage laboratory found that mice housed in an enriched environment (a larger cage with toys, tunnels, and more opportunity for physical activity, learning, and social interaction than in standard housing) have an increased number of new neurons in the dentate gyrus. To determine which element of the enriched environment is critical for the enhanced neurogenesis, mice were assigned to groups with a learning task, wheel running, enrichment, or standard housing. Similar to environmental enrichment, voluntary exercise in a running wheel enhanced the survival of newborn neurons in the dentate gyrus, whereas the other conditions had no effect on cell genesis (Fig. 1). In addition, both enrichment and wheel running led to improved spatial memory function. Other investigators showed that new neurons are generated in the hippocampus after stroke and seizures. These findings have led to the assumption that newborn neurons may be involved in cognition as well as brain repair. However, the conclusions from these studies are based on correlation. The functional role of newborn neurons in the adult brain remains to be determined.

WEB SITES OF INTEREST

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