Genetics of Childhood Disorders: XL. Stem Cell Research, Part 4: Neural Horticulture

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A fundamental scientific principle held for the past 100 years is that children are born with all the neurons they will ever have. New discoveries in stem cell biology and developmental neuroscience are challenging this idea. Because stem cells are self-renewing and unspecialized, they can generate many different cell types. Neuronal stem cells are found within germinal centers in the adult brain such as the subventricular zone and the dentate gyrus of the hippocampus. Scientists have discovered how to harvest these cells from early embryos or the adult CNS for further cultivation in vitro. A variety of genetic, environmental, and molecular factors can then be studied to determine whether they influence the types of cells produced and whether they grow into neurons, heart, muscle, or other cell types. At present, many of the critical factors that regulate their eventual fates are poorly understood. However, new evidence suggests that genomic repair is one of the critical determinants for maintaining stem cells and mature neurons throughout life. This column reviews studies of the role of genomic repair in embryonic and stem cell neurogenesis and differentiation.

Although the interplay between genes and the environment is well established, recent research has found that physical activity can have a striking effect on neurogenesis, neuronal survival, and differentiation in the mature brain. In several studies, voluntary exercise on a running wheel was found to stimulate proliferation of neuronal stem cells in adult rodent dentate gyrus. Physical activity may increase the production of growth factors and other molecules important for neural development. Combinations of several different growth factors have been used successfully to generate dopamine- or serotonin-producing neurons from embryonic stem cells in tissue culture. Once these cells have been propagated and partially differentiated, they may be transplanted into the brain for further study. This type of experiment has been successfully used to treat mice with neurodegenerative disorders of the spinal cord and other brain regions.

Scientists have also made the startling discovery that stem cells are present in the adult body that have the capacity to differentiate into a range of cell types, including blood cells or neurons. Stem cells are not committed to a specific function,

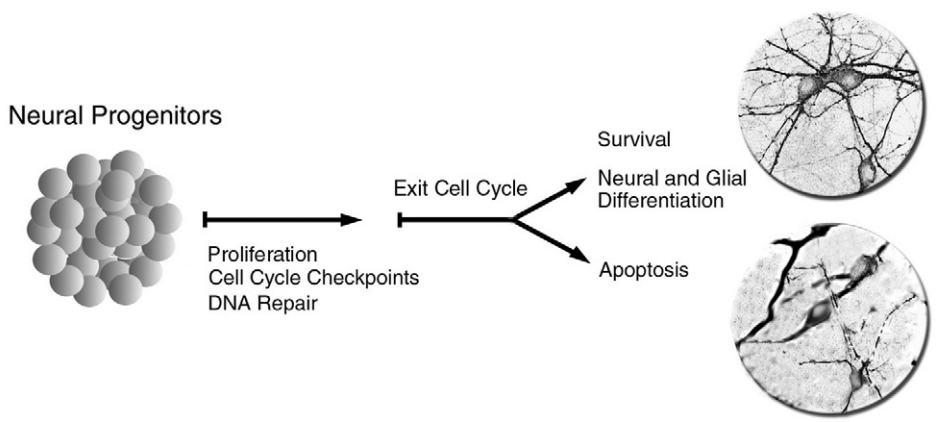

Fig. 1 The fate of CNS progenitors depends on precise regulation of cell proliferation, differentiation, and survival. Recent studies revealed that these processes involve DNA repair. Normally, cell cycle checkpoints allow proliferating neural progenitors to perform necessary genomic repairs. However, when cell divisions are rapid, progenitors may suppress cell cycle checkpoints and damaged regions of DNA may accumulate. It is thought that rapid detection and repair of genetic damage by ATM and nonhomologous end-joining proteins allows these postmitotic cells to survive and begin differentiating into neurons and glia. Unsuccessful repairs of DNA may occur if there is severe DNA damage or defective repair machinery in cells. This will trigger apoptosis, characterized by neurite degeneration, soma vacuolization, and nuclear fragmentation.

as are most cells of the body such as neurons, heart cells, and skin cells. The stem cells present in the body have the unique ability to renew themselves. Beds of neural stem cells reside in the adult brain and can be stimulated to grow in the subventricular zone and dentate gyrus. Harvesting these cells and learning how to propagate them in tissue culture is currently one of the hot test topics of both basic and clinical research because of its potential application in repairing brain injury. Propagating adult neurons from neural progenitors or embryonic stem cells in vitro is now feasible, but much work remains to identify the exact requirements for producing all of the many neuronal cell types present in the adult brain.

Studies are beginning to show that molecules involved in detecting or repairing DNA or chromosome damage also play a critical role in the process of stem cell differentiation and survival (Fig. 1). DNA molecules consist of paired strands of ribonucleotides that are the basis for the genetic code. DNA damage is a serious threat to the integrity of genetic information within the cell. Nicks or single-strand DNA breaks occur commonly and are rapidly detected and repaired by nuclear repair machinery using the intact DNA strand as a template. Cells may also experience a more severe form of DNA damage involving breakage of both DNA strands. These double-strand breaks are extremely dangerous for the cell because they can result in severe chromosomal abnormalities, including translocations and gene amplifications.

Repairing this sort of chromosomal damage, called nonhomologous end-joining, tends to be error-prone because it does not require homologous sequences for rejoining the broken DNA ends. It is a kind of last-ditch effort to repair DNA breaks that occasionally occur in all cells of the body and plays an essential role in suppressing chromosomal aberrations. Apart from this general role, nonhomologous end-joining serves a specific role during maturation of immune cells by creating genetic diversity within immunoglobulin genes. These normal genetic rearrangements are required for the development of humoral and cellular immunity. Loss of this form of DNA processing creates severe combined immune deficiency in which an affected child is unable to produce antibodies required to fight infections.

In nonhomologous end-joining, DNA breaks are repaired in a step-wise manner. This process uses several different proteins including DNA ligase 4, X-ray repair cross-complementation factor 4, and DNA-dependent protein kinase (DNA-PK). Three subunits called Ku70, Ku80, and DNA-PK catalytic subunit comprise the DNA-PK enzyme. DNA double-strand breaks are first detected by Ku70 and Ku80, which bind to the broken DNA and recruit the DNA-PK catalytic subunit to the damage site. The final step in nonhomologous end-joining is the ligation of the broken DNA strands involving DNA ligase 4 and X-ray repair cross-complementation factor 4.

Recent studies in embryonic knockout mice revealed that genes involved in nonhomologous end-joining are required for proper neurogenesis at early stages of brain development. Deficits in nonhomologous end-joining proteins resulted in massive programmed cell death or apoptosis in the embryonic brain, and in some cases the cellular loss was sufficient to cause lethality. Normally, newly born neurons migrate away from where they are born in the ventricular and subventricular zones and begin to extend axons and dendrites. However, in mice deficient for components of nonhomologous end-joining, many of the young neurons begin to undergo apoptosis before they differentiate. The number of apoptotic neurons is extremely high in DNA ligase 4 and X-ray repair cross-complementation factor 4 knockout embryos, and these embryos die during gestation. Ku70/80 knockout embryos have improved chances for survival, but they exhibit significant neurodegeneration at embryonic and postnatal ages, exhibit dwarfism, and die before reaching adulthood. The mildest phenotype is associated with mutations or deletions in the catalytic subunit of DNA-PK, which can result in elevated DNA double-strand breaks and increased neurodegeneration, but normal survival rates.

Many questions remain about the triggers for apoptosis in these and other DNA repair–deficient conditions. Nonhomologous end-joining deficits appear to cause apoptosis of embryonic neurons because of an inability to repair DNA breaks. Therefore, what is the source of DNA strand breaks in embryonic neurons, and do these breaks also appear in differentiating stem cells? Besides external sources such as radiation, DNA damage can result from a high rate of cellular metabolism leading to increased levels of reactive oxygen species that in turn can generate DNA breaks. Such damage is thought to arise during proliferation or periods of high synaptic activity. In actively dividing progenitor cells, DNA breaks would passively accumulate because of suppression of cell cycle checkpoints, which are the intervals when cells normally perform DNA repairs. Once a neuronal progenitor leaves the cell cycle and becomes postmitotic, DNA breaks are detected and repaired continuously. DNA strand breaks in immature neurons could also originate from genetic rearrangements similar to those known to occur in developing immune cells during immunoglobulin gene rearrangements, as this process also requires nonhomologous end-joining proteins. Neuron-specific multigene families are one of the potential candidates for genetic recombination in neurons, but attempts to detect any type of rearrangements within these gene families have failed.

A growing number of genome caretakers are being found that are essential for differentiation and survival of both stem cells and immature neurons. Recently it was shown that telomerase, an enzyme important for protecting the outer arms of chromosomes, is also critical for embryonic neuronal survival. Other genomic caretaker genes have been linked to neurodegenerative disorders of childhood such as xeroderma pigmentosum and trichothiodystrophy. Furthermore, older patients with amyotrophic lateral sclerosis, Parkinson disease, and

Alzheimer disease show reduced DNA repair and hypersensitivity to DNA-damaging agents. Taken together, these findings suggest a relationship between DNA repair deficits and neurodegeneration.

Evidence that neural progenitors and embryonic neurons sustain high levels of endogenous DNA breaks has been found in some strains of knockout mice with deficiencies in DNA repair genes. Our studies of the embryonic brains of these mice found increased activity of caspases, a family of enzymes that act as cellular executioners during apoptosis. At birth, the brains of these mice have fewer differentiated neurons and more neural progenitors compared with wild-type mice. As young adults, when these mice undergo seizures, hippocampal neuronal loss is more substantial compared with wild-type mice of the same strain. Moreover, defective neurogenesis is found in murine embryos deficient for other forms of DNA repair. Taken together, the current evidence suggests that many different types of DNA damage are occurring during neurogenesis and neural differentiation. The range of DNA defects found in dividing precursors and differentiating neurons contradicts the notion that neural development requires a specific form of genetic rearrangement.

What then causes developing neurons and stem cells to be so sensitive to DNA repair deficits? One reason may be that during very rapid proliferation, there is not enough time for a cell to repair its DNA damage. This might create a higher requirement for genomic surveillance and DNA repair compared with when the cell ceases dividing. Another important question was recently addressed: What allows the cell to sense when DNA breaks reach a critical level? A nuclear DNA damage sensor protein called ATM is responsible for sending out a signal when DNA breaks occur. ATM signals to a molecule called p53, which in turn initiates a chain reaction culminating in cell death in an effort to clear away cells with damaged chromosomes. In mice with combined knockouts of nonhomologous end-joining proteins and either ATM or p53, the DNA damage signals to the cell are disrupted, allowing embryonic neurons to escape apoptosis despite having DNA damage. These observations have shed light on the pathogenesis of a childhood disease called ataxia telangiectasia, which is characterized by cancer and neurodegeneration. Mutations in ATM have been found in children affected with this devastating disease. Moreover, mutations of p53 are the most common defect present in many adult human cancers. Once again, mutations in a key gene involved in monitoring DNA breaks is responsible for disease, as cells without the ability to undergo apoptosis are able to survive despite their DNA damage and proceed to proliferate abnormally.

In light of these findings, it is of great interest to know whether DNA caretakers regulate long-term survival of stem cells and neural progenitors. Recent experiments have suggested that ATM-deficient mice also exhibit stem cell deficits. When the brains of ATM-deficient mice were carefully examined in regions such as the dentate gyrus of the hippocampus, researchers did not see the normally observed increase in neurogenesis that is associated with voluntary running. ATM deficits reduce exercise-dependent survival and differentiation of newly generated cells in the adult dentate gyrus. Furthermore, in vitro studies show that ATM-deficient stem cells are unable to undergo the full range of differentiation into multiple cell types. Wild-type adult neuronal stem cells located in the subventricular zone of the cerebral cortex have the capacity to generate neurons, oligodendrocytes, and astrocytes. By contrast, ATM-deficient neural stem cells generate only astrocytes. Like ATM, another enzyme called telomerase is expressed at high levels in dividing neuronal progenitors and immature neurons, but decreases as neurons differentiate. Telomerase prevents chromosomal shortening by adding six-base pair repeats to the chromosomal ends called telomeres. As telomerase levels decrease in both neuronal and non-neuronal cells, rates of proliferation decline because of telomere shortening. Low telomerase levels may account for why transplants of human neuronal precursors cell are often characterized by a progressive decrease in proliferation.

What happens to other aspects of neural differentiation and maturation when DNA integrity is compromised by DNA repair deficits? Random alterations in the genome lead to genetic mutations in important secreted signaling molecules or cell surface receptors that reduce the ability of a young neuron to respond to signals in its environment. Delayed or inappropriate responses to environmental signals would disrupt migration, synapse formation, or activity-dependent differentiation. Perhaps milder DNA repair deficits are a contributing factor in the spectrum of human developmental disorders, including mental retardation or other cognitive disorders.

There are still many uncertainties about how, when, and where DNA repair serves a critical role in neuronal survival. It is clear, however, that the stem cell field will be fertile ground for answering these and other questions. A better understanding of the requirements for DNA repair in stem cells, neural progenitors, and immature neurons may also aid development of strategies for harvesting and transplanting neural progenitors to treat neurological disorders.

WEB SITES OF INTEREST

Stem cells—a primer: http://www.nih.gov/news/stemcell/primer.htm
Ataxia telangiectasia Web site (including AT children's project and others): http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/gene?ATM
DNA repair: http://www.ultranet.com/~jkimball/BiologyPages/D/DNArepair.html

ADDITIONAL READINGS

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