Commentary

Cell Cycle Events in Neurons

Proliferation or Death?

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Classically, we are taught that neurons are terminally differentiated and lack the ability to divide. The extreme rarity of brain tumors of neuronal origin, coupled with the failure of oncogenic transformation to create cell lines, seems to confirm this assertion. However, recent findings indicate that isolated neurons *in vitro* can, in the right environment, apparently undergo multiple rounds of proliferation.¹ Nonetheless, although the dogma of absolute neuronal cell cycle dormancy has been revoked *in vitro*² and plasticity in mitocompetency thus retained to some degree, we should not rush to alter the textbooks. Indeed, in this issue of *The American Journal of Pathology*, Migheli and colleagues³ present a convincing case for alterations in the mitotic machinery in vulnerable neurons in the *weaver* mouse that appear to contribute to, if not directly cause, cell death.³

The *weaver* mouse is so named because selective degeneration of cerebellar neurons generates motor deficits in homozygotes and presents principally as instability of gait. In the cerebellum of the *weaver* mouse, the apparently normal granule cell precursors fail to differentiate, migrate to the internal granule cell layer after birth, and actually die in the external germinal layer.⁴ Purkinje's cells and neurons in the deep cerebellar nuclei also degenerate,^{5,6} and neuronal degeneration also occurs in the midbrain, where subsets of dopamine-containing neurons are vulnerable between postnatal days 7 and 21.^{7,8} Phenotypically, a dopamine signaling abnormality causes mild locomotor hyperactivity in this mouse; male sterility attributed to the death of the majority of spermatids after puberty is another notable abnormality.^{9,10} Despite the variety of symptoms, the *weaver* condition results from only a single gene mutation, *wv* mutation, which arose spontaneously over 30 years ago.¹¹ Although the *wv* mutation is a recessive mutation, heterozygous mice actually exhibit mild cellular abnormalities but no obvious behavioral abnormalities and become sterile only after 4 months of age.¹²

The *wv* gene maps to murine chromosome 16, a region homologous with the Down's syndrome region of human chromosome 21,¹³ and has been identified as a missense point mutation in the *Girk2* gene that results in a glycine-to-serine substitution in the pore-forming region of G-protein-coupled inwardly rectifying K⁺ channel subunit 2 (*GIRK2*). The channel consists of *GIRK2* and a related subunit, *GIRK1*. The *weaver* mutation in *GIRK2* causes a loss of potassium selectivity and G-protein sensitivity, which further leads to chronic depolarization and massive cell death.¹⁴ Given the rather specific cell degeneration in the *weaver* mouse, it is surprising to find that *GIRK2* is expressed throughout the central nervous system and testis.¹²

The reason some cell types are more susceptible to *wv* mutation remains a mystery. In fact, it is still unknown how these cells die. One hypothesis is that disruption of cell movement, which results in ectopic location and thus incorrect projection/connection, contributes to neuronal cell death in *weaver* mouse.⁵ However, in this issue, Migheli et al³ provide convincing evidence that a cell cycle abnormality occurring before migration may be the cause of cell death. These findings add to the growing body of evidence suggesting that cell cycle disturbance, including inappropriate re-entry into the cell cycle in, eg, neurodegenerative diseases such as Alzheimer's disease (AD) and failure to exit the cell cycle as normal (eg, *weaver* mouse), results in neuronal cell death.

The cell cycle is a highly regulated process with numerous checks and balances that ensures a homeostatic balance between cell proliferation and cell death in the presence of appropriate environmental signals. The tight regulation of cell cycle control may intrinsically require an intimate coupling of the cell death mechanism in order to remove defective cells during the cell cycle, such that cell cycle checkpoints appear to link the cell cycle to apoptosis.¹⁵ The cell cycle is typically divided into four phases: G1, S (DNA replication), G2, and M (mitosis),

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and it is the sequential expression and activation of cyclin/cyclin-dependent kinase (cdk) complexes, the main regulators of cell cycle progression, that orchestrate the transition from one phase to another.¹⁶ Cells can exit the cell cycle to enter and stay at resting (G0) phase, which is the case in terminally differentiated cells. The expression/activation of cyclin D/cdk4,6 complex, triggered by the presence of mitotic growth factors, controls the re-entry of resting G0 cells into the G1 phase of cell cycle.¹⁷ Thereafter, the G1/S transition is controlled by the activation of the cyclin E/cdk2 complex¹⁷ such that absence of cyclin E and/or the inhibition of the cyclin E/cdk2 complex by p21, p27, and p53 will cause the cell cycle to be arrested at the G1 checkpoint.¹⁶ The fate of the G1 arrested cells depends on the presence or absence of cyclin A, and only in the absence of cyclin A can the cells return to G0 and redifferentiate. Otherwise, cells die via an apoptotic pathway.¹⁸ The DNA replication in the S phase and the transition to the G2 phase is regulated by the activation of cyclin A/cdk2 complex and proliferating cell nuclear antigen (PCNA).¹⁶ The main regulator of the G2/M transition is the cyclin B/cell division cycle 2 complex.¹⁶ Any perturbation of these regulators will result in the arrest of the cell cycle at G2/M transition point. Cells arrested at G2/M checkpoint lack the ability to redifferentiate and die via an apoptotic pathway.¹⁸ Therefore, if a terminally differentiated cell, for any reason, reaches late G1 or even later, when cyclin A is expressed, arrest will lead to cell death.

A number of neurodegenerative disorders, such as *stagger*, *lurcher* in mice¹⁹ and Pick's disease, intractable temporal lobe epilepsy, progressive supranuclear palsy, Lewy body disease, and Parkinson's disease in humans,^{20–22} are associated with a dysregulated cell cycle, as manifested by a re-entrant mitotic phenotype. Evidence is emerging in AD that associates a mitotic phenotypic profile with select degeneration of neurons. For example, cytoskeleton alterations, hyperphosphorylation of tau, and the expression, phosphorylation, and metabolism of β -precursor protein (β PP) as well as the re-emergence of select cyclins and their cognate kinases in degenerating neurons in AD are, in fact, all features of cycling cells.²³ Notably, the induction of Ras and Cdc42/Rac signal transduction pathway is involved in the abnormality of cell cycle events in AD such that various components of the cell cycle machinery are apparently activated in vulnerable neurons in AD.^{24,25} Indeed, the reappearance of cyclin D, cdk4, and/or Ki67 in all the cases reported suggests that the neurons in AD are no longer quiescent. The appearance of cyclin E/cdk2 complex reflects that neurons may have emerged from G0,²¹ whereas the aberrant expression of cyclin B1/cdc2 complex indicates the degenerating neurons in AD may reach G2 phase at least in some cases.^{21,26} Because mitotic figures have never been observed in the neurons of AD or normal brain and premature activation of cdc2 triggers a mitotic catastrophe leading to apoptosis, it seems that the reappearance of cyclin B1/cdc2 complex reflects an apoptotic-type cascade rather than mitosis.^{27,28} Additionally, the up-regulation of p21 and p16^{29,30} may reflect the attempt of the cell to retain cell cycle control in re-

sponse to ectopic mitogenic stimuli. However, one cannot exclude intrinsic abnormalities in the genesis of cell cycle re-entrant event(s). Examples include the concurrent expression and aberrant localization of PCNA and cyclin B³¹; the concurrent appearance of cdk4 and p16³⁰; and the presence of cyclin E and cyclin B but absence of cyclin D and cyclin A.²¹ All of these abnormalities point to an inadequate or failed control of cell cycle in these neurons. The lesson here may be that *in vivo* neuronal re-entry into cell cycle is intrinsically dysregulated and will inevitably be arrested due to the incompetent signaling or sensitivity to this signaling. Because a variety of cell cycle disturbances may result in arrest in various phases, apoptosis can then have multiple entry points from the cell cycle, wherein different cell-cycle components will be expressed in apoptosis with different initiators.³² Indeed, this aspect may account for the apparent inconsistency of the reported cell cycle markers expressed in vulnerable neurons in different neurodegenerative disorders, or even in the same complex disorder. In fact, Busser et al³¹ reported up-regulation of PCNA and cyclin D in some AD cases, while Nagy et al²¹ found no differences. This may further reflect the complexity of the initiation of cell cycle events in AD. The broad parallels between some neurodegenerative diseases and the early processes during oncogenesis in other tissues raise the question why a postmitotic neuron would die if it attempted to divide. Heintz³³ has suggested that upon receiving a cell division signal, the signal transduction pathway in terminally differentiated cells is switched to an alternative effector pathway that leads to cell death rather than proliferation. Indeed, the apparent expression of cell cycle markers in these cells suggests that the signal has triggered an aberrant cell division process, which itself may have toxic sequelae in terminally differentiated cells and cause their death. For example, mitochondria proliferation before mitosis would pose an oxidative threat to the cell arrested at G2/M checkpoint, which possibly could not be overcome, and the cellular compensations would remain inadequate. There are, indeed, significant increases in mitochondria in vulnerable neurons in AD and such a mechanism could account for the profound oxidative damage found in AD.³⁴

In *weaver* mice, it is clear that the cause of the pathology is the mutation in GIRK2. Given the finding of Migheli et al,³ there is still a great gap in our understanding between GIRK2 mutation and failure to exit the cell cycle. There may be a regulator of cell cycle and cell death that can monitor the functional state of the granule cells and serves as a kind of surveillance molecule, mediating the exit from the cell cycle in normal cells, and the hyperpolarization, prevented by mutated GIRK2 in homozygous mouse, may be a necessary part of the signal for a cell to exit the cell cycle. Such a surveillance mechanism is likely to be selected by evolution. In AD, it is a similar but a far more complex story. It has been shown that there are at least three genes, amyloid precursor protein and presenilins 1 and 2, associated with early-onset AD, but little is known about the relation between the re-entry of the cell cycle and these mutations. Our working hypoth-

esis is that these mutations may accelerate the G1 progression block, thus causing arrest in the late G1 phase and cell death in the end.²³ What factors stimulate the neurons to re-enter the cell cycle is still unknown. The inappropriate expression or release of a growth factor or factors is a likely explanation which is supported by the findings of elevated level of some growth factors in AD.²³

The *weaver* mouse can be studied to elaborate the mechanisms of cell cycle stasis and subsequent cell death, furthering the understanding of cell cycle re-entry mechanisms in neuronal degeneration. Because a number of neurodegenerative diseases are associated with a re-entrant mitotic phenotype, this model may provide a useful tool to study cell cycle dysfunction and its sequelae in primary neurons. The elucidation of the mechanisms involved in failed cell cycle control would permit interruption of the natural course of this chronic neurodegeneration.

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