**Linking Cell Cycle Reentry and DNA Damage in Neurodegeneration**

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Aberrant cell cycle activity and DNA damage have been observed in neurons in association with various neurodegenerative conditions. While there is strong evidence for a causative role for these events in neurotoxicity, it is unclear how they are triggered and why they are toxic. Here, we introduce a brief background of the current view on cell cycle activity and DNA damage in neurons and speculate on their relevance to neuronal survival. Furthermore, we suggest that the two events may be triggered in common by deregulation of fundamental processes, such as chromatin modulation, which are required for maintaining both DNA integrity and proper regulation of cell cycle gene expression.

Key words: cellular activity; neurodegeneration; DNA damage

Aberrant Cell Cycle Activity in Neurodegeneration

Aberrant neuronal expression of numerous cell cycle proteins, such as cdc2, cdk4, cyclins B1 and D, p16, and Ki-67, were observed in Alzheimer’s disease (AD) brains over 10 years ago, as covered in previous reviews. In addition to aberrant expression of cell cycle genes, neurons in AD brains can undergo DNA replication activity, as evidenced by DNA incorporation of the thymidine analogue bromodeoxyuridine, and extra copies of some chromosomal loci. This collective phenomenon, often referred to as cell cycle reentry, is both unexpected and perplexing, considering that most of these neurons have been terminally differentiated during development and had been quiescent for decades. The cell cycle activity appears to be abortive, and actual division of neurons has not been observed.

DNA Damage in Neurodegeneration

DNA is constantly subjected to damage, such as chemical modifications (for example 8-oxoguanine, a type of oxidative DNA modification) as well as single- and double-strand breaks. These can arise endogenously from attack by intracellular metabolites (such as reactive oxygen species) or through errors during DNA replication (e.g., stalled replication fork), or exogenously through exposure to carcinogens or irradiation. DNA damage poses a threat to metazoans as it can cause mutations ranging...
from base pair changes to chromosomal translocations to even aneuploidy, which can result in neoplasia.\textsuperscript{7}

There has been a steadily accumulating body of evidence of damage to DNA in various neurodegenerative conditions, since first suggested over two decades ago.\textsuperscript{8} For example, upregulation of DNA strand breaks in neurons have been reported in AD\textsuperscript{9} and HD,\textsuperscript{10} while increased 8-oxoguanine lesions have been reported in ALS\textsuperscript{11} and ischemia.\textsuperscript{12} Damage to mitochondrial DNA has been reported in PD.\textsuperscript{13}

Interestingly, it was demonstrated that oxidative DNA damage accumulated in the human brain with age, and could be responsible for the gradual loss of neuronal function observed with old age.\textsuperscript{14} As neurodegenerative diseases, such as AD, PD, and ALS are highly age-dependent, this finding raises the possibility that accumulation of DNA damage constitutes the elusive “age component” that may trigger, contribute to, or permit neuronal pathogenesis.

### Relevance of Neuronal Cell Cycle Activity and DNA Damage in Neurodegeneration

Thus, both aberrant cell cycle activity and DNA damage in neurons are observed during the progression of multiple neurodegenerative conditions. An important issue is whether these play a causative role in neuronal death or are simply parallel phenomena observed during neurotoxicity. The collective evidence supports an active role for cell cycle activity and DNA damage in neuronal death.

Expression of cell cycle proteins has been reported as a predictor for neuronal death, for example in AD postmortem brains and in a mouse model displaying neurodegeneration.\textsuperscript{15,16} In addition, overexpression of oncogenes or cell cycle proteins, such as SV40 large T’antigen, c-myc, or E2F-1, which drive cell cycle activity, has been shown to be toxic to neurons in vivo and in culture.\textsuperscript{17–19} Furthermore, inhibition of cell cycle machinery, for example through cdk inhibitors, has been shown to be protective against neurotoxic insults, such as ischemia and kainate-induced excitotoxicity.\textsuperscript{20,21}

There is also significant evidence for an active role for DNA damage in neurodegeneration. The importance of DNA integrity in neurons is exemplified by congenital conditions, such as ataxia telangiectasia, xeroderma pigmentosum, Werner syndrome, and Cockayne syndrome, in which the underlying genetic defect is in genes that are involved in DNA repair.\textsuperscript{22} In these conditions, following relatively normal brain development, progressive and severe neuronal death is observed, demonstrating that maintenance of DNA integrity is a requirement for neuronal survival at the postdevelopmental stage. Furthermore, it is established that neurons are highly susceptible to toxicity from DNA damaging agents, such as camptothecin, etoposide, and hydrogen peroxide.\textsuperscript{23}

### Why Do Aberrant Cell Cycle Activity and DNA Damage Occur in Neurons, and Why Are These Events Neurotoxic?

In metazoans, maintaining proper control of cell cycle activity and DNA integrity is paramount for preventing neoplasia. This is especially relevant in actively proliferating cells, and is reflected in the high level of “evolutionary investment” in terms of complex cell cycle related machinery and robust DNA damage repair/response mechanisms found in these cells, as reviewed previously.\textsuperscript{7} It is important to note that cell cycle control, DNA damage, and cancer are intricately interlinked in the context of cycling cells. Multiple checkpoints and tumor suppressor mechanisms, such as p53, act to halt cell cycle progression if DNA damage is detected. Conversely, DNA damage is an important trigger for mutagenesis and chromosomal rearrangements, and subsequent alterations in oncogenes or tumor suppressor genes can elicit uncontrolled cell cycle activity and
cancer. Importantly, as an antineoplastic safeguard, cells that have excessive DNA damage and/or attempt to progress through the cell cycle while harboring DNA damage will be eliminated through apoptosis.\(^7\)

In contrast, neurons are postmitotic and highly differentiated, and it would appear at first glance that cell cycle activity and DNA damage would have limited relevance and consequence, especially compared with actively proliferating cells. Interestingly, neurons appear to have decreased baseline levels of certain types of DNA repair processes, possibly reflecting decreased demand for DNA repair due to absence of cell cycle activity and decreased risk of neoplastic consequences.\(^24,25\) Decreased repair capacity combined with the inherently high metabolic rate of neurons (and increased byproducts that can damage DNA) may lead to accumulation of DNA damage over time. This process may be exacerbated or accelerated in neurodegenerative conditions, such as AD.\(^25\)

Thus, accumulation of DNA damage may reflect an evolutionary strategy where energy expenditure is invested toward neuronal function rather than DNA integrity.\(^25,26\) On the other hand, it is more difficult to resolve the underlying purpose for aberrant cell cycle reentry in neurons. Perhaps, as discussed below, aberrant cell cycle activity in neurons may be better viewed as neglected/lost suppression of cell cycle activity (and cell cycle gene expression) rather than an active purposeful event, in a similar manner to neglected DNA maintenance.

A remaining question is why DNA damage and cell cycle activity are toxic to neurons. From an evolutionary point of view, even differentiated cells may still have some capacity to form neoplasia. For example, while the expression of oncogenic SV40 large T antigen can trigger neuron death,\(^17\) some groups have successfully immortalized differentiated neuron-like cells by overexpressing this gene.\(^27,28\) Thus, if DNA damage is combined with aberrant cell cycle activity, namely S phase, it may still be prudent for the organism to eliminate these neurons via programmed cell death. At the very least, this neuronal cell death may represent a leftover effect of antineoplastic safeguards that are critical in cycling cells.

Along these lines, it has been hypothesized that both DNA damage and cell cycle activity are required to trigger apoptosis in adult neurons in neurodegenerative conditions.\(^25\) It has been observed that neurons can tolerate certain types of DNA damage without triggering cell death. However, it is predicted that DNA damage coupled with cell cycle activity will potently trigger checkpoint responses that will induce neuronal death.\(^25\) In support of this notion, neuroprotective effects of inhibiting genes with checkpoint function, such as p53 and ataxia telangiectasia mutated (ATM), have been reported.\(^29,30\)

### Linking Cell Cycle Reentry and DNA Damage in Neurodegeneration

As discussed, cell cycle reentry and DNA damage are features of multiple neurodegenerative conditions that may act in concert to trigger neuronal death. However, only recently has a link between the two phenomena been suggested in the context of neurodegeneration. It was reported that DNA damage can induce cell cycle reentry in primary neurons.\(^29\) Furthermore, DNA damage and cell cycle reentry have been observed concomitantly in culture and mouse models displaying neuronal death,\(^31,32\) although these are not necessarily linked to common neurodegenerative conditions, such as AD.

Cyclin-dependent kinase 5 (Cdk5) is a nonconventional cdk that is primarily active in postmitotic neurons and is hyperactivated following accumulation of its regulatory binding partner p25, during neurodegenerative conditions, such as AD and stroke.\(^33,34\) We have previously described an inducible p25 overexpression model that displays several features of AD including neuronal death, amyloid beta upregulation, tau hyperphosphorylation and neurofibrillary tangles, and cognitive decline.\(^35-37\)
In this model, we observed that both cell cycle activity and double-stranded DNA breaks occur in neurons, in a highly associated manner both spatially and temporally.\textsuperscript{38}

How are DNA damage and cell cycle reentry linked? A likely contributing factor is that one may positively impact on the other. As mentioned, DNA damage can trigger neuronal cell cycle activity and death, but paradoxically, in a checkpoint-dependent manner.\textsuperscript{29} Conversely, cell cycle activity, in particular DNA replication activity, in neurons may cause DNA damage. It is known that S phase is a period of particularly high susceptibility to DNA strand breaks, for example through stalling or collapse of replication forks.\textsuperscript{39} At a molecular level, tumor suppressor/DNA damage response genes may link aberrant cell cycle activity and DNA damage. For example, ATM and p53 can serve as DNA damage checkpoints and suppress cell cycle activity in response to DNA damage, but can also activate DNA repair mechanisms.\textsuperscript{7} Thus, loss of function of such genes is often associated with DNA damage and loss of DNA integrity in proliferating cells. Interestingly, loss of function of ATM in ataxia telangiectasia causes progressive neurodegeneration.\textsuperscript{22}

Another possibility is that both aberrant cell cycle activity and DNA damage can be triggered by deregulation of fundamental cellular processes that are required for both DNA integrity and proper control of cell cycle gene expression. An intriguing candidate process is altered chromatin modulation and transcriptional deregulation, which is already known to be relevant in the context of neurodegenerative disease.\textsuperscript{40,41}

In eukaryotes, nuclear DNA is wrapped around histone octamers, which collectively are referred to as nucleosomes, the basic unit of chromatin. Modifications to histones, such as acetylation and methylation, affect the degree of DNA/histone affinity and internucleosomal affinity, resulting in “open” and “closed” (or “silent”) loci of chromatin.\textsuperscript{42} DNA at these open loci is highly accessible to molecules that interact with DNA, including transcription factors and DNA damaging agents. Thus, through effects on DNA accessibility, the regulation of chromatin plays an important role in a variety of processes in the cell.

Alteration of chromatin can affect cell cycle activity through altered expression of genes involved in the cell cycle. For example, opening of chromatin leading to increased transcription of cell cycle machinery can drive cell cycle activity, or conversely, increased expression of cell cycle inhibitors can inhibit cell cycle in proliferating cells.\textsuperscript{43} Differences in effects may depend on the specific loci that are affected as well as the cellular context. In the case of adult neurons, deregulation of chromatin, in particular opening of cell cycle gene loci that are normally maintained as silent, may result in aberrant cell cycle gene expression and cell cycle activity.

Furthermore, open chromatin loci are thought to be more accessible to DNA damaging agents.\textsuperscript{44} For example, it has been shown that inhibition of histone deacetylases (HDACs), which leads to hyperacetylation of histones and opening of chromatin, renders DNA hypersensitive to damaging agents, such as gamma and UV irradiation.\textsuperscript{45} Furthermore, chromatin modulation is involved in DNA repair.\textsuperscript{46}

Thus, as tight regulation of chromatin is thought to play a role in a wide range of cellular processes,\textsuperscript{42} regulation of this process may elicit deleterious consequences which include deregulation of cell cycle activity and loss of DNA integrity. We recently uncovered a pathological pathway which supports this notion. In the p25 overexpressing model for neurodegeneration, p25/Cdk5 inhibited HDAC1 activity, resulting in aberrant cell cycle activity and formation of DNA double strand breaks, and neurodegeneration.\textsuperscript{38} HDAC1 inactivation-induced cell cycle protein expression appears to be a direct result transcriptional derepression, while further studies are required to ascertain how HDAC1 inactivation results in double-strand breaks. This study demonstrates that neurotoxic stimuli can impact chromatin regulation to cause neurodegeneration. Other chromatin
modulators that potentially may be deregulated in neurodegenerative disorders and other diseases include the other HDACs and histone methylases/demethylases.

In conclusion, the purpose and underlying mechanisms of neuronal cell cycle activity and DNA damage are still poorly understood phenomena, but they likely work in concert to induce neuron death in multiple neurodegenerative conditions. A general overview of the process is outlined in Figure 1. The identification of common underlying mechanisms may have important therapeutic implications.

Conflicts of Interest

The authors declare no conflicts of interest.

References


