DNA damage response and neuroprotection

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1. ABSTRACT

The protection of genomic integrity is a major challenge for living cells that are continuously exposed to endogenous and environmental DNA-damaging insults. To cope with the consequences of DNA lesions which interfere with essential DNA-dependent processes including transcription and replication, cells are equipped with an efficient defense mechanism termed the DNA damage response. Its function is to eliminate DNA damage through DNA repair and to remove cells with incurred DNA damage by apoptosis. The DNA damage response has been investigated mainly in proliferating cells, in which the cell cycle machinery is integrated with the DNA damage signaling. Our recent studies suggest that the cell cycle machinery is involved in DNA damage response of postmitotic neurons. Given a high metabolic rate, continuous exposure to oxidative stress and extensive gene transcription activity, the importance of the DNA damage response and the integrated cell cycle signaling for maintaining genomic stability in neurons cannot be overemphasized. The suppression of cell cycle activation is considered neuroprotective, especially in experimental models of stroke. The present review discusses the importance of DNA damage response for postmitotic neurons and the mechanisms of its dysfunction leading to different neurodegenerative disorders. In this regard, a better understanding of the mechanisms underlying DNA damage response in neurons may have important therapeutic implications for different neurodegenerative diseases.

2. DNA DAMAGE RESPONSE

One of the cellular macromolecules that are highly affected by intracellular as well as extracellular insults is DNA. More than 10^4 DNA damaging events occur in each mammalian cell every day. They may be induced by environmental stresses such as chemical pollutants, ultraviolet light, and ionizing radiation (1, 2). Intracellular DNA damaging insults include spontaneous DNA depurinations, replication errors and oxidative stress formed in the course of normal aerobic metabolism and by other enzyme systems concerned with maintaining redox stability as sources of reactive oxygen species (ROS). DNA damage can be part of normal genomic transactions, such as meiotic recombination and the maturation of the immune system genes via V(D)J recombination (3, 4). The maintenance of genome integrity after DNA damage is vital for the continued proliferation, transcription and survival of eukaryotic cells. For this reason, all organisms have evolved a number of distinct DNA repair systems allowing them to cope with various kinds of damage: mismatch repair, base excision repair (BER), direct damage reversal, nucleotide excision repair, and double strand break (DSBs, the most cytotoxic DNA lesion) repair that can be further divided between homologous recombination and non-homologous end-joining (NHEJ) (5-7). All eukaryotic cells have evolved a multifaceted response to counteract the potentially deleterious effects of DNA damage. DNA damage is sensed by a highly conserved mechanism which involves protein kinases such as ataxia-telangiectasia mutated/ataxia-telangiectasia and

Rad3-related (ATM/ATR) to recognize DNA lesions and activate cell cycle checkpoints that in turn, trigger both transcriptional and transcription-independent responses, including activation of DNA repair machinery and cell-cycle arrest. The DNA damage response involves multiple levels of regulation, affecting not only DNA repair genes but also genes that influence protein and lipid turnover, cytoskeleton remodeling, and general stress pathways (8).

2.1. DNA damage response and neurodegeneration

Neurons are extremely active cells and metabolize up to 20% of the oxygen that was consumed by the organism. Under normal physiological conditions, synthesis of ATP in mitochondria results in the production of various ROS as by-products. In addition, neurons have low levels of antioxidant enzymes and subsequently a lower capacity to neutralize the ROS (9). For this reason and due to the relatively reduced capacity for cellular regeneration compared with other organs, brain cells are believed to be particularly susceptible to the damaging and highly toxic effects of ROS. Chronic exposure to ROS has been discussed as a major risk factor for neurodegenerative disease (10). Markers of oxidative stress are found in postmortem examination of brains from patients with many neurodegenerative disorders, including Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (11). On the other hand, ROS are particularly important genotoxic agents. Aging is accompanied by a decline in mitochondrial function and associated increase in oxyradical production (12, 13). These age-related effects may provide a unifying mechanism that can explain the fact that normal aging is the most reliable and robust risk factor for neurodegenerative diseases. DNA strand breaks have been reported in neurons after reperfusion of ischemic tissue, well in advance of DNA fragmentation caused by the apoptotic process (14, 15). Recent studies of patients with AD, PD, ALS, stroke, and Huntington's disease suggest that oxidative stress and neuronal DNA damage are common features of these diseases (16, 17, 64). DNA damage is an important initiator of neuronal cell death and has also been implicated in neurodegenerative conditions. DNA damage caused by ROS includes altered bases, abasic sites, and single- and double-strand breaks which can be prevented by DNA repair. A complex defense mechanism, which acts as a guardian of genome integrity involves not only DNA repair and its coordination with other cellular processes such as cell cycle progression in mitotic cells but also eliminating cells with incurred DNA damage via apoptosis (18-20). Thus, the DNA-damage response is actually a complex network of signaling pathways that affects many aspects of cellular metabolism after the induction of DNA damage. Hallmarks of this response are the activation of cell-cycle checkpoints and the appropriate DNA repair pathways, which lead to cellular survival or, in certain contexts, initiation of apoptotic program. The DNA damage response is essential for both mitotic and postmitotic cells, and defects in various branches of this response lead to severe neurological demise (for review see 21-23). In contrast to dividing cells, non-dividing cells may not need to repair the bulk of their genome (24). Transcription-coupled repair is a mechanism that maintains the integrity of active genes in non-dividing cells. Its molecular details are not clear, but it is generally assumed that RNA polymerase II serves as a lesion sensor and attracts repair enzymes when stalled by a DNA lesion (25).

DNA integrity is essential for survival, and for both DNA replication and transcription. DNA damage can have different consequences on transcription including a mutation in the mRNA potentially resulting in a non-functional or unstable protein (24). DNA lesions can block the progression of RNA polymerase II (25), which results in a deficit in the required protein and is a strong signal for apoptosis (24). The common output of this transcriptional disturbance is a lack of essential proteins that results in cellular dysfunction and cell death (26). Examination of various types of differentiated cells reveals that DNA repair in differentiated cells is attenuated at the global genome level, but maintained in expressed genes (9).

A primary step in the DNA damage response is efficient detection of the type of DNA damage. Dependent upon the type of damage, different protein repair complexes are involved in the overall cellular response. Once the DNA damage or modification is registered by the cell, a signaling cascade is triggered which slows down or arrests cell-cycle progression. The overall function of this arrest (checkpoints) is to recognize damaged or abnormally structured DNA and to coordinate cell-cycle progression with DNA repair, enabling repair mechanisms to correct the genetic lesions before they are passed on to the next generation of daughter cells (27,28). Thus, the DNA damage response allows cells to keep DNA damage under control. If the damage is too severe, cells undergo apoptosis or senescence. If the damage is repairable, the cell will stall in the cell cycle to enhance fidelity of the DNA repair process. Failure of cells to respond to DNA damage is a primary event associated with mutagenesis and environmental toxicity (29-31). The importance of DNA repair for neuronal fate decision is illustrated by the fact that a reduced DSB repair in neurons from SCID mice deficient in DNA-PK, a critical element of DSB repair (77), is associated with hypersensitivity of SCID neurons to DSB-induced apoptosis (32, 33). This reinforces idea that the failure of DNA repair machinery is linked to the onset of apoptosis (29-31).

2.2. Genomic instability syndromes and neurodegeneration

Genetic defects in essential elements of the DNA damage response network lead to genetic disorders termed 'genomic instability syndromes' (7). Cells with inherited defects in the DNA damage response are more susceptible to the harmful impact of DNA damage, including defects in checkpoints and accumulation of mutations that may lead to cell transformation. It is not surprising that defects in various branches of the DNA damage response result in severe neurological demise that is illustrated by neurodegeneration accompanying human hereditary disorders (34-36). Progressive neurodegeneration has been described in one of such disorders, ataxia telangiectasia (AT; 23). AT has been extensively studied for its cancer predisposition and neurodegeneration. AT is characterized

by increased DNA DSBs caused by mutations in the ATM, the primary mobilizer of the DSB response in mammalian cells and the nuclear protein kinase, which phosphorylates key players in the various arms of this network (23, 37). Once activated, ATM triggers a phosphorylation signaling cascade leading to activation of checkpoints (Chk1 and Chk2) and subsequent phosphorylation of late effector proteins such as E2F1, p53 and CDC25 family members, which inhibit cell cycle progression, activate DNA repair systems, or induce apoptosis if the DNA damage is too extensive (6). Thus, ATM orchestrates a signaling network consisting of repair mechanisms, cell cycle checkpoints, and apoptotic pathways that lead the cell to repair and survival, or apoptosis. Mutation of ATM causes defective cell cycle checkpoint activation, a reduced capacity for repair of DNA DSBs and abnormal apoptosis, all of which contribute to the major features of AT including genome instability, increased cancer risk and neurodegeneration (38). ATM deficient cells are hypersensitive to DNA DSB inducers (39) and more resistant to DSB-initiated apoptosis (40-44). The amount of unrepaired DNA after γ-irradiation which induces DSBs is greater in cells from AT-patients than in cells from normal individuals (45). One of the major features of ATM deficiency is a striking resistance of neurons to DSB-induced apoptosis (40-44). For example, mice with targeted deletions in the ATM gene have dramatically reduced neuronal apoptosis in response to genotoxic damage (46, 86), suggesting that ATM is essential for apoptotic signaling in neurons to eliminate these cells when DNA damage is non-repairable. On the other hand, ATM deficiency leads to a reduced DNA repair capacity (45). This increased sensitivity to DNA damage due to the impairment of the DNA damage response that engages ATM signaling might lead to the accumulation of genetic lesions that eventually compromise cellular function and viability. The occurance of progressive neurodegeneration in AT patients makes obvious that ATM(-/-) neurons die. However, since apoptotic signaling is compromised in these cells, neurodegeneration in AT patients might be caused by nonapoptotic mechanism mediated by the accumulation of unrepaired DNA damage. In contrast to AT patients, ATM-knock-out mice which exhibit most of the characteristics of human AT, barely show the cerebellar degeneration (46, 47). However, human-like neurodegeneration was observed in mice in which NBS1, a member of the MRN (Mre11/Rad50/Nbs1) complex, a DSB sensor that is involved in the initial processing of the breaks and is required for ATM activation (49,50) was knocked out in the nervous system (48). This phenotype can be explained by the requirement of the MRN complex for activation of both ATM and ATR (49, 50). Thus, the abrogation of these two key factors of the DNA damage response in the nervous system might increase an effect in the murine cerebellum. The importance of ATM in DNA DSB response of neurons is supported by the existence of functional ATM-mediated damage response in another type of differentiated cells, myotubes (51). Together, these notions underscore the role of DNA damage response in neuronal cell fate suggesting that ATM transduces the DNA damage response in differentiated cells similarly to in proliferating cells (52).

Another common type of ataxia is ataxia-oculomotor apraxia type 1 (AOA1) caused by aprataxin (APTX) mutation (53). Cells deficient in aprataxin are sensitive to agents that cause single strand breaks in DNA, and this protein has been shown to associate with DNA repair proteins (54). Reduced DNA ligation activity was observed in *APTX*-disrupted chickens' DT40 cells and astrocytes from Aptx$^{-/-}$ mice. Thus, a major role of aprataxin appears to be in the resolution of abortive DNA ligation intermediates (55). A family of sun-sensitive human diseases, Xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) also are characterized by neural dysfunction. Patients with CS and TTD suffer from progressive mental deterioration. Certain of the XP groups also show a spectrum of neurodegeneration involving neuronal loss and mental deterioration (16, 56). Most of the initial studies on neuronal DNA repair have focused on the nucleotide excision repair (NER) pathway given the neurological defects observed in XP and CS patients caused by mutations in the components of the NER system. Presumably, the neuronal cell death found in XP and CS patients is not due to exogenous sources, but is due to the lack of ability to repair endogenous DNA damage (16, 56). Thus, defects in the DNA damage response network are an important initiator of neuronal cell death implicated in neurodegenerative conditions.

2.3. DNA damage response and cell cycle activation in differentiated neurons

In cycling cells, the DNA damage response is comprised of cell cycle arrest at specific checkpoints, presumably to allow time for the damage to be repaired, or to activate apoptotic program if the damage is too extensive to be repaired (58). Thus, cell cycle regulation is integrated with the DNA repair mechanisms, and these signalings even use some common proteins (19). Adult neurons are terminally differentiated cells that are excluded from the cell cycle (G0 quiescent state). Neurons have been considered to be "locked" into the G0 phase of the cell cycle. Normally, the release of a cell from the resting G0 phase results in its entry into the first gap phase (G1), during which the cell prepares for DNA replication in the S phase. This is followed by the second gap phase (G2) and mitosis (M). Accumulating evidence suggests that postmitotic neurons re-express cell cycle markers with the occurrence of apoptotic neuronal cell death (59, 60). While terminally differentiated neurons retain the ability to reactivate the cell cycle, they rarely progress to mitosis and neuronal proliferation and typically induce apoptosis instead (59, 61, 62). These neurons undergo full or partial DNA replication, showing that they have entered the S phase (43, 30) followed by cell death, not cell division (for review see 20; 64, 65). In addition, in a variety of disorders, cell cycle proteins are expressed in neurons undergoing apoptosis. These disorders include stroke (64), ALS (66), PD (67), AD (68-71), Nieman-Pick type C disease (72), and traumatic brain injury (73). The functional relevance of cell cycle machinery to neuronal apoptosis was demonstrated by utilizing genetic manipulations of components of the cell cycle. The adenoviral delivered kinase-dead mutant form of CDK4 protected cultured
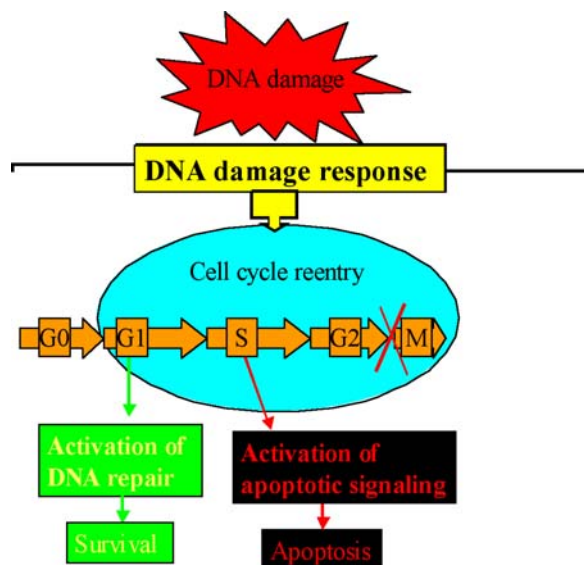
Figure 1. Potential roles for the cell cycle machinery in DNA damage response of neurons are illustrated. DNA damage induces the DNA damage response and cell cycle reentry. The G0-G1 transition activates DNA repair. If DNA damage is not repairable, neurons enter S phase which activates apoptotic signaling.

cerebellar granule neurons from hypoxia mediated apoptosis (64). Neurons derived from mice expressing kinase dead CDK4 or null for its regulator cyclin D1 are resistant to hypoxia mediated ischemic death (64). Further support for the functional role of cell cycle pathway in ischemic neuronal death is provided by observations that cortical neurons and cerebellar granule neurons derived from E2F1 (a factor critical for cell cycle progression) null mice are less susceptible to death mediated by glucose and oxygen deprivation- and kainate induced neuronal death (74,75). Blocking cell cycle-associated E2F-1 transcription protected cultured dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPTP) toxicity, and E2F-1-deficient mice were significantly more resistant to MPTP-induced dopaminergic cell death than their wild-type littermates (76). E2F1 deficiency improved the recovering of CA1 neurons from loss of synaptic transmission following anoxic insult of hippocampal slices *in vitro* (74). The simultaneous silencing of CDK4 and CDK6 by RNA interference technique protected cultured cortical neurons against hydrogen peroxide (H_2O_2)-induced apoptosis (77). These data support the idea that cell cycle reentry underlies neuronal apoptosis *in vivo* as well as *in vitro*. A direct link between cell cycle and neuronal death was made with the observation that CDC2, a cell cycle regulator, induced the phosphorylation and activation of BAD, a trigger of apoptosis (78).

The DNA replication process has been suggested to be lethal for neurons (60). Thus, the DNA replication machinery itself has been found to trigger cell death and generate a death signal in neurons (79). In contrast to proliferating cells, neurons that enter the S phase before they die by apoptosis, fail to express DNA polymerase-α

(DNA pol-α), which is essential for the canonical DNA synthesis (79). Neurons instead overexpress DNA pol-β that is crucial for base excision repair (80). In base excision repair, pol β is capable of filling small gaps and nicks in DNA and acts in an error-prone fashion (81, 82). Up-regulation of pol β in mammalian cells has been found to increase spontaneous mutagenesis (83). It raises the possibility that in neurons entering S phase, a pol β-directed DNA replication might produce additional DNA damage, thus contributing to the execution of apoptotic death (84).

Little is known about the mechanisms of DNA damage response in terminally differentiated neurons, especially about its integration with cell cycle machinery. However, there is both *in vitro* and *in vivo* evidence of a link between DNA damage and cell cycle reentry in dying postmitotic neurons. Analysis of the X-harlequin (Hq) mutation in the gene encoding apoptosis-inducing factor which is accompanied by oxidative stress, has demonstrated that in affected mice, many cerebellar granule cells had newly synthesized nuclear DNA (evidence of cell cycle reentry) and were positive for oxo8dG, a marker of oxidative DNA damage. Oxidative DNA damage was noted in many neuron types of these mice (84). The Hq mice develop progressive ataxia beginning at 4–5 months of age. The onset of ataxia is correlated with apoptosis of cerebellar granule cells (84). These data suggest the association between DNA damage, cell cycle activation and apoptosis in neurons. Using flow cytometry and BrdU incorporation analyses, we have demonstrated that cell cycle activation followed by apoptosis is induced by DNA damage and can be blocked along with the DNA damage response (43). Suppression of the ATM, a key component of DSB DNA damage response, pharmacologically (by administration of caffeine and wortmannin) or by using mouse neuronal cell cultures lacking ATM (-/-), attenuated both apoptosis and cell cycle reentry (43), suggesting that both cell cycle activation and apoptosis are constituents of the DNA damage response in neurons and that the involvement of cell cycle in neuronal apoptosis signaling is a unifying feature of proliferating cells and neurons. In support of this view, Alvira *et al.*, 2007 (44) recently demonstrated that caffeine not only attenuated apoptosis of cerebellar granule neurons induced by the neurotoxin MPP+ but prevented expression of cyclin D and the transcription factor E2F-1, essential for cell cycle reentry of postmitotic neurons. In contrast to these data, Yang and Herrup, 2005 (85) demonstrated the reappearance of cell cycle markers in at-risk striatal neurons in both human and mouse AT. One explanation for these divergent findings is that another essential for DNA damage response factor such as ATR may function in AT and initiate the expression of cell cycle markers in the absence of functional ATM. However, numerous studies demonstrated significant impact of ATM deficiency on DSB repair, cell cycle signaling in proliferating cells and on apoptosis in neurons. ATM deficiency compromised apoptotic signaling in postmitotic neurons (for review see 23, 38). Therefore activation of cell cycle signaling in conditions of ATM deficiency cannot be associated with programmed cell death (apoptosis) since ATM is a key

component of the DNA damage response and is involved in both DSB repair and DSB-initiated apoptosis in postmitotic neurons. Thus, data of Yang and Herrup, 2005 (85) suggest that non-apoptotic cell death in AT is accompanied by cell cycle activation which is unlikely. In addition, Yang and Herrup, 2005 (85) did not specify the type of neuronal death in AT which is very important in this context since several studies have demonstrated that AT- associated neurodegeneration is not associated with apoptotic cell death but rather is a consequence of accumulating genetic lesions and eventual loss of neuronal viability by another cell death mechanism (38, 39, 45). Moreover, ATM deficiency results in resistance to DSB-induced apoptosis (40-44, 46, 86). In the *Hq* mutant mouse, many dying neurons abnormally reentered into the cell cycle, other neurons that degenerate did not. Neurons which did not activate the cell cycle were not positive for oxo8dG, a marker of oxidative DNA damage, nor activated caspase-3, suggesting a non-apoptotic type of cell death. Electron microscopy also revealed that these cells underwent necrosis rather than apoptosis (84). These findings are concordant with a premise of association between DNA damage response, apoptosis and cell cycle activation in neurons. AT is caused by loss of ATM function. The major function of ATM is the DNA damage response in proliferating as well as differentiated cells (50-52). In proliferating cells, the DNA damage response is important for both DNA repair and apoptosis. We and others demonstrated that ATM deficiency results in compromised apoptotic signaling in neurons (40-44). This observation can be linked to findings on the essential role of cell cycle activation in neuronal apoptosis (64, 66-73, 76). In support, the suppression of cell cycle signaling attenuates neuronal apoptosis (43, 64, 73, 76, 77), including apoptosis initiated by DNA damage (43, 44, 72, 73). Given the importance of ATM in neuronal DNA damage response and DSB-initiated apoptotic signaling (19, 40-44, 52), as well as an essential role of cell cycle activation in neuronal apoptosis, we suggest that cell cycle activation in neurons is associated with DNA damage response, and ATM plays a role in activation of the cell cycle machinery in neurons in response to DSBs. This suggestion is supported by direct evidence that ATM deficiency results in attenuation of both cell cycle activation and apoptosis (43, 44). Although these observations imply that induction of unscheduled cell cycle reentry is highly correlated with, and is likely induced by DNA damage, the mechanisms of DNA damage response, its link to cell cycle machinery and the roles of this machinery in DNA damage-initiated neuronal apoptosis remain to be defined.

3. DNA DAMAGE RESPONSE AND NEUROPROTECTION

DNA damage is an important initiator of neuronal apoptosis. Recent studies of patients with AD, PD, ALS, stroke, and Huntington's disease suggest that neuronal DNA damage are common features of these diseases (16, 17, 64). Therefore, studies on DNA damage and repair pathways in neurons have more than a basic science application; they may help in understanding how neurons repair DNA damage and the effect of unrepaired

DNA on neuronal death and pathogenesis of human diseases including AD, PD, ALS, and cancer and may have therapeutic implications.

Another application of the understanding of toxic DNA damage mechanisms in neurons in the clinical arena relates to the neurotoxicity associated with antineoplastic therapy which is based on genotoxins such as γ-irradiation and chemical genotoxins. Among the numerous side effects of cancer treatments, neurotoxicity that includes both neurocognitive dysfunction and peripheral neuropathy, occurs frequently (87-90). The antifolate drug, methotrexate, which produces DNA damage is known to induce mental retardation and seizures in children treated against acute lymphoblastic leukemia (91). While cancer patients with neurocognitive and peripheral neuropathy manifestations produced by antineoplastic therapy have been documented, the underlying mechanism and the role of DNA repair in preventing this neuropathy have not been well studied (92). It has been found, however, that at least some of antineoplastic treatments including methotrexate, γ-irradiation and etoposide are toxic for neurons *in vitro* (43).

Thus, the understanding the mechanisms underlying DNA damage response in neurons may have important therapeutic implications for different neurodegenerative diseases and neurotoxicity associated with antineoplastic therapy.

3.1. DNA damage signaling and neuroprotection

Oxidative damage to neuronal genomic DNA, a common feature of various neurodegenerative diseases, consists of different lesions including hydroxyl radical-modified bases, apurinic/apyrimidinic abasic site lesions, single-strand breaks, and DSBs (17, 93, 94). Accumulation of oxidative DNA lesions in the brain, if not repaired promptly, may trigger cell death (94, 95). The results from a number of studies have suggested that blockage of DNA damage-triggered apoptosis signaling pathways can offer remarkable neuroprotection (96-100).

The base-excision repair (BER) pathway is one of critical mechanisms for repair of oxidative DNA lesions in the brain (101-103) and its activation might have a neuroprotective effect. It has been found that hypothermic treatment is able to promote BER activity, and attenuate the levels of oxidative DNA lesions after ischemia (104). The hypothermic treatment also suppressed poly(ADP-ribose) polymerase-1 (PARP-1) and the tumor suppressor and transcription factor p53, critical factors in DNA damage response signaling (105). Therefore, the decrease in oxidative DNA damage by preconditioning which promotes DNA repair may contribute to the attenuation of neuronal death after focal ischemia and reperfusion. Recent studies on the preconditioning suggest that this strategy enables brain cells to be markedly more resistant to subsequent severe ischemia (106-108).

The suppression of key factors of DNA damage response such as PARP or p53 has been reported to produce neuroprotective effect (109, 110). ROS and

associated DNA damage generated during ischemia-reperfusion injury cause PARP activation. PARP is involved in DNA repair machinery, however, massive DNA damage leads to overactivation of PARP-1 and depletion of intracellular nicotinamide adenine dinucleotide (NAD^+), essential in initiation of the electron transport chain, and ATP thereby compromising mitochondrial function. These factors may cause necrotic cell death (111) or activate the Bax-dependent apoptosis pathway (97,112). Pretreatment with PARP inhibitor, 8-hydroxy-2 methyl-quinazolin-4-[3H]one (NU1025) restored cell viability to approximately 73% and 82% in H_2O_2 and 3-morpholinosyndnomine (SIN-1, a peroxynitrite donor) injured cells, respectively. *In vivo*, NU1025 reduced total infarct volume up to 45%, when administered before reperfusion. NU1025 also produced significant improvement in neurological deficits. These results demonstrate a significant neuroprotective effect of NU1025 and suggest its potential in cerebral ischemia (113). Other PARP inhibitors were also neuroprotective both *in vivo* and *in vitro* (114-116).

p53 production is rapidly increased in neurons of the injured brain tissue in response to a range of insults, including oxidative stress produced by cerebral ischemia and experimental traumatic brain injury (110, 117). Preclinical data suggest that agents that inhibit p53 such as pifithrin-alpha (PFT) may be effective therapeutics for several neurodegenerative conditions including ischemia and experimental traumatic brain injury (110, 118).

ATM plays an essential role in DNA damage response of both proliferating and differentiated cells such as neurons (see for review 21-23). Its deficiency causes a striking resistance of neurons to DSB-induced apoptosis (40-44). Caffeine, an ATM inhibitor, has been shown to attenuate apoptosis of cerebellar granule neurons induced by neurotoxin MPP+ (44), as well as cortical neurons exposed to etoposide (43). However, caffeine cannot be considered neuroprotective because ATM deficiency compromises DNA repair and thereby leads to accumulation DNA lesions (45) that eventually compromise cellular function and viability. This view is supported by the fact that progressive neurodegeneration is seen in AT patients that are the deficient in ATM (23). Since apoptotic signaling is compromised in ATM deficiency conditions, neurodegeneration in AT patients might be caused by nonapoptotic mechanism mediated by the accumulation of unrepaired DNA damage.

3.2. Neuroprotection provided by blocking cell cycle reentry

Recently, we and others demonstrated that DNA damage may activate cell cycle machinery in postmitotic neurons (43, 44, 73, 77, 84). Accumulating evidence suggests that DNA damage contributes to the loss of neurons in various neurological disorders (12, 13, 44, 73). The suppression of cell cycle activation is known to be neuroprotective *in vitro* and *in vivo* (111, 119, 120). In a PD model, blocking cell cycle-associated E2F transcription protected cultured dopaminergic neurons against MPTP toxicity. E2F-1-deficient mice were significantly more

resistant to MPTP-induced dopaminergic neuron death (76). Thus, cell cycle inhibitors may have important implications for therapeutic use.

Cyclin-dependent kinases (CDKs) play an important role in cell cycle regulation (121). CDK inhibition by flavopiridol, olomoucine and roscovitine has been shown to induce neuroprotective effects in the *in vivo* and *in vitro* stroke models (119-122). However, these inhibitors have multiple cellular targets including non-CDK-related kinases and are not specific (123). Recently, we demonstrated that CDK4 and CDK6 knockdown produced by RNA interference (RNAi)-based technique significantly protected neurons against hydrogen peroxide-induced apoptosis (77). PD 0332991, a highly specific inhibitor of CDK4 and CDK6, also produced neuroprotective effect *in vitro* (I.I Kruman, unpublished results). Like flavopiridol, PD 0332991 has been developed for the treatment of cancer. This potent antiproliferative agent has been shown to be effective in tumor regression in mice (123). Another antineoplastic agent which presently being used in a human trial in the cancer clinic, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (PAN-811 or Triapine[TM]) exerted neuroprotective effect *in vivo* (124). PAN-811 inhibited expression of cell cycle markers in cultured neurons exposed to H_2O_2 (E.I. Schwartz, unpublished results). Thus, these CDK inhibitors may have important therapeutic implications for neurodegenerative disorders.

4. CONCLUSIONS AND PERSPECTIVES

In summary, maintenance of genomic stability is highly dependent on the DNA damage response, an extensive signaling network that is rapidly activated and modulates numerous cellular processes (23). The DNA damage response is essential for postmitotic neurons, and defects in various branches of this response lead to severe neurological demise (see for review 21-23). Since DNA damage contributes to the observed loss of neurons in various neurological disorders (16, 17, 44, 73), the DNA damage response may be involved in neurodegeneration. The importance of the DNA damage response in neuronal survival is illustrated by the existence of 'genomic instability syndromes' caused by genetic defects in essential elements of DNA damage response (7) and by neurodegeneration accompanying these human hereditary disorders (7, 34-36, 125). Several laboratories have demonstrated that cell cycle proteins are expressed in neurons undergoing apoptosis. The expression of cell cycle markers has been demonstrated in different neurodegenerative disorders (64; 66-73). The functional relevance of cell cycle machinery in neuronal apoptosis was demonstrated in experiments utilizing the suppression of cell cycle components by genetic or pharmacological manipulations. The suppression of different cell cycle components resulted in protection of neurons against apoptosis initiated by H_2O_2, kainate, MPTP, and hypoxia both *in vitro* and *in vivo* (43, 44, 64, 74-77). A direct link between cell cycle and neuronal death has been found in activation of apoptotic trigger, BAD by the cell cycle regulator CDC2 kinase (78). Since DNA damage

contributes to the loss of neurons in various neurological disorders (12; 13) and neuronal apoptosis *in vtro* (43, 44, 77), and cell cycle machinery is involved in apoptotic signaling, the DNA damage response may play an important role in apoptotic and cell cycle signaling in neurons. Accumulating evidence suggests this view. The suppression of different factors involved in DNA damage response such as PARP, p53 has neuroprotecvtive effect. ATM deficiency leads to a striking resistance of neurons to DSB-induced apoptosis *in vitro* and *in vivo* (40-44, 46, 86), suggesting that ATM is essential for apoptotic signaling in neurons to eliminate these cells when DNA damage is non-repairable. However, ATM deficiency is associated with a reduced DNA repair capacity (45) and neurodegeneration in AT patients (38), most likely caused by the accumulation of unrepaired DNA damage that eventually compromises cellular viability which is not associated with apoptotic signaling. Accumulating evidence supports the functional relevance of cell cycle machinery to neuronal apoptosis including evidence of neuroprotective effects produced by the suppression of cell cycle signaling (43, 64, 73, 76, 77). In this regard, a link between DNA damage response and cell cycle reentry in dying postmitotic neurons remains to be further elucidated although several studies suggest that both cell cycle activation and apoptosis contribute to the DNA damage response in neurons and that the involvement of cell cycle machinery in neuronal apoptosis signaling is a unifying feature of postmitotic neurons and proliferating cells (43, 44, 84).

An essential role of cell cycle activation as well as neuroprotective effect of the suppression of cell cycle signaling have been demonstrated in a variety of neurodegenerative disorders (44, 64, 66-73,76), suggesting that the cell cycle components may provide an effective therapeutic target.

However, the question remains: what happens to DNA damage that is not repaired in neurons? Accumulation of DNA lesions in the genome leads to a loss in the fidelity of information transferred from DNA to proteins and to the transcription of defective proteins that eventually leads to cell death (126). But if postmitotic, differentiated neurons re-enter the cell cycle, their unrepaired DNA may trigger cell death due to the accumulation of DNA damage since DNA replication might produce additional DNA damage (84, 126). The mechanisms by which a neuronal cell is forced into apoptosis versus DNA repair also remain vague. It is likely that this signaling is managed by the DNA damage response.

While the role and mechanisms of cell cycle regulation of neuronal apoptosis are just beginning to be characterized, the fundamental question of why postmitotic neurons engage cell cycle machinery to control apoptosis and how this cell cycle machinery relates to DNA damage response remains to be the subject of considerable speculation and future studies.

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Abbreviations: BER: base excision repair; NHEJ: non-homologous end-joining; PARP: poly (ADP-ribose) polymerase; p53: tumor suppressor and transcription factor p53; AT: ataxia telangiectasia; ATM: ataxia telangiectasia mutated; pol-α: DNA polymerase-α; DNA pol-β: DNA polymerase-β; CDK: cyclin-dependent kinase.

Key Words: Neurodegeneration, Neuroprotection, Cell cycle, DNA damage, DNA repair, Apoptosis, Review

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