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Forum Review

The Contribution of the DNA Damage Response to Neuronal Viability

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ABSTRACT

Neurons are extremely active cells and metabolize up to 20% of the oxygen that was consumed by the organism. Despite their highly oxygenic metabolism, neuronal cells have a lower capacity to neutralize the reactive oxygen species (ROS) that they generate or to which they are exposed. High levels of ROS can lead to accumulation of damage to various cellular macromolecules. One of the cellular macromolecules highly affected by intracellular as well as extracellular insults is DNA. Neurons are also highly differentiated, postmitotic cells that cannot be replenished after disease or trauma. Since neurons are irreplaceable and should survive as long as the organism does, they need elaborate defense mechanisms to ensure their longevity. This review article mainly focuses on certain mechanisms that contribute to neuronal longevity, and concentrates on the DNA damage response in neuronal cells. The various mechanisms of DNA repair are briefly described, and focus is on those mechanisms that are activated in neuronal cells following DNA damage. Evidence is presented to show that proper DNA damage response is critically important, not just for normal neuronal development but throughout the entire life of any organism. Defective DNA damage response in older human age can generate neurodegenerative disorders such as Alzheimer's or Parkinson diseases. *Antioxid. Redox Signal.* 9, 211–218.

INTRODUCTION

NEURONS ARE UNIQUE CELLS: they are postmitotic, highly differentiated, and can not be replenished after disease or trauma. Neurons are highly active, being the main transmitter of electrical and chemical signals, and in their transcriptional profile. Since neurons are irreplaceable and should survive as long as the organism does, they need elaborate defense mechanisms to ensure their longevity. The amount of oxygen consumed by the brain far exceeds its size relative to the rest of the body organs. These active physiological roles coupled with relatively high oxygen consumption expose the neurons to a stressful environment. One of the cellular macromolecules that is highly affected by intracellular as well as extracellular insults is DNA. It has been estimated that each cell in our body undergoes 20,000 DNA lesions every 24 h. This number is probably higher because of the extremely high neuronal cell mitochondrial activity. The many types of DNA lesions that result from these insults are rapidly detected, with subsequent activation of an intricate web of signaling pathways known as the *DNA damage respons*e. This response culminates in activation of cell cycle checkpoints and the appropriate DNA repair pathways, or, in certain contexts, initiation of apoptotic programs. The DNA damage response is a hierarchical process that is executed through a series of steps. DNA lesions are detected by *sensor* proteins that recognize either the lesions themselves or chromatin alterations that may follow the DNA damage. *Transducers* are brought into action to convey the damage signal to downstream *effector*s. It is this relay system from transducers to effectors that enables a single DNA lesion to modulate numerous pathways. The transducers might also be involved in the assembly of DNA repair complexes at the sites of DNA damage (63) (Fig. 1).

We show here that a proper DNA damage response is critically important not just for normal development of the nervous system, but throughout the life of the organism. Proper maintenance of genome integrity is critically important for

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normal aging while malfunction of the DNA damage response can accelerate neuronal death and lead to neurodegenerative diseases.

DNA REPAIR IN THE NERVOUS SYSTEM

Terminally differentiated cells do not replicate their genomic DNA. Thus, as long as they are able to maintain the integrity of the genes that must be expressed, they can therefore dispense with the task of removing DNA damage from the nonessential bulk of their genome. There is increasing experimental evidence that this is indeed the case, at least for some repair pathways such as nucleotide excision repair (NER). Examination of a number of terminally differentiated cell systems revealed that DNA repair is attenuated at the global genome level, but maintained in expressed genes. How these cells manage to repair transcribed genes is not fully elucidated, but there are indications that the transcription-coupled repair (TCR) pathway could maintain integrity of the transcribed strand (TS) in the active genes. The nontranscribed strand (NTS) of active genes in neurons is also well repaired, a phenomenon that has been named differentiation-associated repair (DAR). It is conceivable that DAR is required to maintain the integrity of the template strand that is needed by TCR to complete the repair of lesions in the TS of essential expressed genes with high fidelity (11).

One of the critical issues in modern pathobiology is how cells govern the decision to live or die, and the cost of making such a decision. Nowhere are these questions more poignant than in deciphering the tissue-specific responses to DNA damage. Mutations in DNA repair enzymes, malfunctions in cell cycle regulation, and genetic instability are associated with most somatic cancers. However, in many hereditary diseases arising from mutations in DNA repair proteins, the same dominant mutations that cause cancer in dividing cells are often associated with cell death in terminally differentiated neurons (26).

Vemuri *et al*. (62) tested whether the DNA repair enzyme, DNA-dependent protein kinase, plays an important role in the survival of cerebral cortical neurons in mice. In mice with the SCID (severe combined immunodeficiency) mutation, DNA-PKcs is truncated near the kinase domain, which causes loss of kinase activity. They compared the spatial and temporal aspects of neuronal cell death in SCID versus isogenic wild-type embryos and found a significant increase in dying cells in SCID mice evidenced by nuclear changes, DNA fragmentation, and caspase-3 activity. Additional biochemical and immunocytochemical studies indicated that of several DNA repair enzymes investigated, only poly(ADP-ribose) polymerase (PARP) was increased in SCID mice, possibly in response to elevated DNA strand breaks (62). To assess the role of DNA-PK deficiency in the maturation and survival of neurons, primary neuronal cultures derived from the cerebral hemispheres of newborn wild-type and SCID mice were prepared. Purified neuronal cultures developed comparably in terms of neurite formation and expression of neuronal markers, but SCID cultures showed a significant increase in the percentage of dying cells. Furthermore, when apoptosis was induced by staurosporine, SCID neurons died more rapidly and in higher numbers. Apoptotic SCID neurons exhibited nuclear condensation, DNA fragmentation, and caspase-3 activation, but treatment with the general caspase inhibitor did not prevent staurosporine-induced apoptosis. Thus, it was concluded that a DNA-PK deficiency in cultured SCID neurons may cause an accumulation of DNA damage and increased susceptibility to caspase-independent forms of programmed cell death (9). Knockout animals for components in the nonhomologous end joint DNA repair (NHEJ) pathway demonstrate a distinct pattern of cell death in the developing brain. It was demonstrated that cell death was also present in the developing retina of E14.5 Ku86-deficient mouse embryos, suggesting that the increase in cell death in the retina is associated with chromosome breaks. In the adult retina, the investigators failed to find continuing apoptosis, but interestingly, the number of total neuronal cells was decreased (29).

Merlo *et al*. found that granule cell primary cultures and cerebellar organotypic slices can integrate and express a promoter-less GT vector, thus revealing DNA repair activity in postmitotic neurons. Furthermore, they showed that this activity increases after a mild apoptotic stimulus and correlates with increased expression of both HR and NHEJ DNA repair factors (44).

THE DNA DAMAGE RESPONSE AND NEURODEVELOPMENT

The differentiation of cells in the nervous system, as in other organs, is the consequence of a complex program that directs the cascade of events leading to hundreds of different types of fully differentiated neuronal cells. Proper DNA damage response is critically important for normal neural development. Interestingly, during this process the number of neuronal cells produced far exceeds the final number of mature neurons. One of the most important processes responsible for removing superfluous neurons is programmed cell death. Substantial death of migrating and differentiating neurons occurs within the developing CNS of mice that are deficient in genes required for repair of double-strand DNA breaks. These findings suggest that large-scale, previously unrecognized, double-strand DNA breaks occur normally in early postmitotic and differentiating neurons; and that cell death occurs if the breaks are not repaired. Despite the significant impact of their occurrence, the cause and natural function of such breaks remain a mystery. Massive cell death of migrating of migrating and differentiating neurons was observed in XRCC4 and ligase-IV deficient mice. These proteins appear to be involved in the final step of the DNA recombination process, completing repair of the DNA double helix by closing the DSB (reviewed in Ref. 46). Gao *et al*. further stressed the importance of proper DNA repair in lymphogenesis and neurogenesis when they showed that XRCC4-deficiency causes a pleiotropic phenotype that includes embryonic lethality and massive neuronal apoptosis (22). Gao *et al*. further showed that p53-deficiency rescues several aspects of the XRCC4-deficient phenotype, including embryonic lethality, neuronal apoptosis, and impaired cellular proliferation.

However, there was no significant rescue of impaired V(D)J recombination or lymphocyte development (21).

Herzog *et al*. (25) reported resistance to apoptosis in the developing central nervous system (CNS) of Atm −/− mice after ionizing radiation, leading to the suggestion that during development Atm might assume a role of a proapoptotic protein. This lack of death occurred in diverse regions of the CNS, including the cerebellum, which is markedly affected in ataxia-telangiectasia (A-T). In wild-type but not Atm −/− mice, upregulation of p53 coincided with cell death, suggesting that Atm-dependent apoptosis in the CNS is mediated by p53. Further, p53 null mice showed a similar lack of radiation-induced cell death in the developing nervous system. Atm may function at a developmental survival checkpoint that serves to eliminate neurons with excessive DNA damage (25). Lee *et al*. (39) further showed that Atm-dependent apoptosis occurs at discrete stages of neurogenesis: Atm-dependent apoptosis occurred in γ-irradiated mouse embryos only in the postmitotic populations that were present in the neuroepithelial subventricular zone of the developing nervous system. Notably, Atm deficiency did not prevent radiation-induced apoptosis in multipotent precursor cells residing in the proliferating ventricular zone. Atm-dependent apoptosis required p53 and coincided with the specific phosphorylation of p53 and caspase-3 activation (17). The hallmark of A-T is progressive severe neurodegeneration. New findings have suggested that in addition to a role for Atm in responding to DNA damage in the nervous system, it could also be important for adult neurogenesis (43). As mentioned earlier, inactivation of certain DNA repair genes such as DNA ligase IV results in massive neuronal apoptosis and embryonic lethality in the mouse, indicating the occurrence of endogenously formed DNA double-strand breaks during nervous system development. Lee *et al.* (39) report that Atm is required for apoptosis in all areas of the DNA ligase IV-deficient developing nervous system. However, Atm deficiency failed to rescue deficits in immune differentiation in DNA ligase IV-null mice. These data indicate that Atm responds to endogenous DNA lesions and functions during development to eliminate neural cells that have incurred genomic damage (39). Ligase IV deficiency induced apoptosis seems to be dependent on p53 activation. Frank *et al*. showed that p53 deficiency rescued embryonic lethality, neuronal apoptosis, and fibroblast proliferation/senescence defects but not lymphocyte development or radiosensitivity (19).

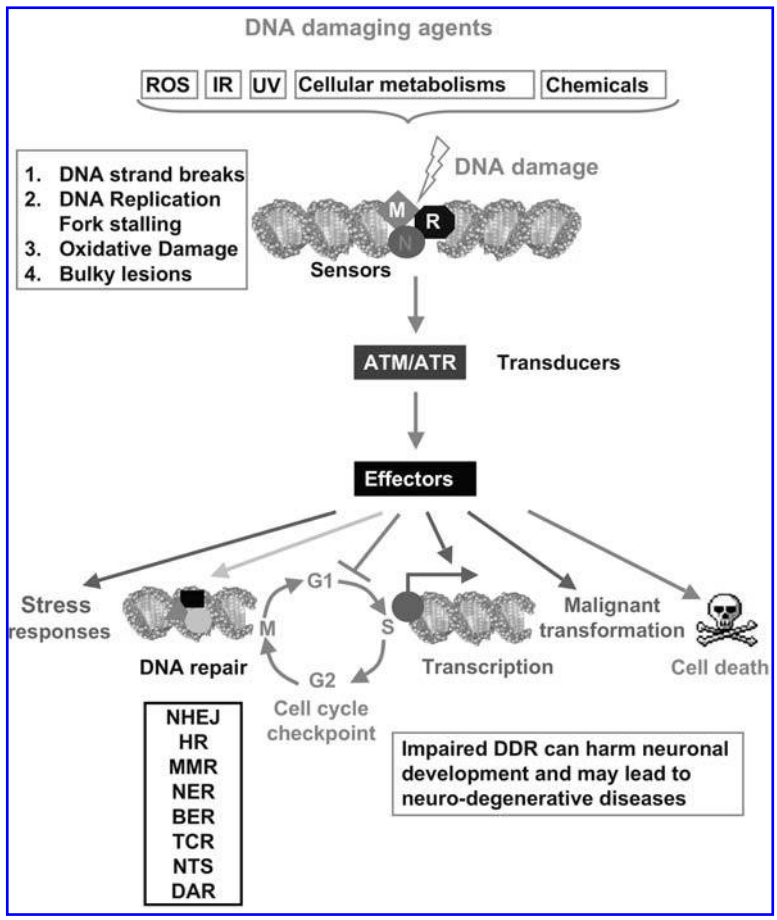

FIG. 1. Schematic representation of the DNA damage response (DDR) activated in response to various incidents of DNA damage. Nuclear DNA can be damaged by various agents, such as reactive oxygen species (ROS), ionizing radiation, UV light, various cellular metabolites, and various types of chemicals. These DNA damaging agents can cause various hits, such as bulky lesions that mainly affect the nucleotides and bases. High levels of oxidative metabolites can lead to the formation of 8-oxo-guanine, DNA replication errors or staling. These agents can also cause single-strand breaks and the most cytotoxic DNA damage, double-strand breaks (DSB). Strand breaks are firstly detected by various sensors such as the MRN complex (Mre11, Rad50, Nbs1), which in turn activate transducer kinase proteins such as ATM and ATR. These kinases are capable of phosphorylating a large number of effector proteins that convey the message and activate various cellular processes. These include the activation of vast range of stress responses. DNA repair mechanisms [DSBs can be repaired either by the error prone nonhomologous end joint (NHEJ) or by homologous recombination (HR) that occurs mainly in proliferating cells. Affected bases or nucleotides can be repaired by mismatch repair (MMR), nucleotide excision repair (NER), and base excision repair (BER)]. In proliferating cells, proper DDR activation will inhibit the progression of the cell cycle machinery and, in parallel, activate the transcription of specific genes. Replication of damaged DNA can also lead to malignant transformation. If the DNA damage exceeds the DNA repair capabilities, the damaged cells will activate their apoptotic programs. Proper DDR is critically important, not just for normal neuronal development but throughout the entire life of any organism. Defective DNA damage response in older human age can generate neurodegenerative disorders such as Alzheimer's or Parkinson's diseases.

Although Atm-deficient mice recapitulate most of the A-T symptoms, they do not display cerebellar pathology, one of the most devastating symptoms of A-T. It was recently shown that the protein core of the MRN complex composed of Mre11, Rad50, and Nbs1 serves as a sensor for DNA damage (3, 33, 58). Recently, Frappart *et al*. (20) reported that conditional inactivation of the NBS1 gene in mouse neural tissues resulted in a combination of the neurological anomalies characteristic of NBS, A-T, and A-T-like disorder (A-TLD), including microcephaly, growth retardation, cerebellar defects, and ataxia. Loss of Nbs1 causes proliferation arrest of granule cell progenitors and apoptosis of postmitotic neurons in the cerebellum. Furthermore, Nbs1-deficient neuroprogenitors show proliferation defects and contain more chromosomal breaks, which are by ATM-mediated p53 activation. Remarkably, depletion of p53 substantially rescued the neurological defects of Nbs1 conditional mutant mice (20).

THE DNA DAMAGE RESPONSE IN AGING

Compelling evidence in the literature points to a central role of accumulation of DNA damage in the aging process of postmitotic cells such as neurons in the central nervous system (CNS) (31, 34). However, the aging process does not affect the CNS uniformly (23), and various brain regions and types of neurons differ substantially in the amount of DNA damage accumulation during aging (50). Specifically, more DNA damage was found in the aging hippocampus than in the aging cerebellum (40). A substantial increase in the amount of DNA single-strand breaks in hippocampal pyramidal and granule cells and in cerebellar granule cells but not in cerebellar Purkinje cells was observed. The reverse pattern was found for age-related reductions in total numbers of neurons: the total number of cerebellar Purkinje cells was significantly reduced during aging, whereas the total numbers of hippocampal pyramidal and granule cells cerebellar granule cells were not (51).

DNA DAMAGE RESPONSE IN NEURODEGENERATIVE DISEASES

One of the most devastating symptoms of old age is neurodegenerative disease. Adamec *et al*. (1) investigated the presence of DNA damage in Alzheimer's disease (AD), utilizing independent assays for three different types of DNA strand breaks. Sections from hippocampi of AD brains, brains with Alzheimer neurofibrillary changes (Ch) from nondemented individuals, and controls (C) were labeled with (a) the TUNEL assay to identify blunt-ended and three protruding termini of breaks in double-strand DNA, (b) the Klenow assay to detect single-strand and double-strand breaks with protruding five termini, and (c) the Apostain assay, which utilizes a monoclonal antibody to single-strand DNA and is based on the decreased stability of apoptotic DNA to thermal denaturation caused by DNA breaks. The highest incidence of nuclei positive for either molecular form of DNA strand breaks was detected in AD, followed by Ch and controls C (1), demonstrating that sick neurons bear damaged DNA that cannot be properly repaired. The hallmarks of AD brains are deposition of A-beta plaques, appearance of neurofibrillary tangles, and extensive loss of neuronal cells. Interestingly, evidence of DNA breaks could be detected prior to the appearance of tangles, which are one of the hallmarks of AD (45). An evolutionarily conserved protein complex, containing Rad50, Mre11, and Nbs1 is involved in diverse aspects of genome metabolism, including DNA damage recognition, DNA end processing and repair, and checkpoint activation (30, 47, 56). All these components are essential for survival in mammalian cells. Hypomorphic mutations in the human MRE11 and NBS1 genes cause A-TLD and Nijmegen breakage syndromes (NBS), respectively (26, 55). Jacobsen *et al*. (27) compared the levels of the Mre11 complex proteins in brain samples from AD and age-matched nondementia controls and showed for the first time that the Mre11 complex proteins are present in neurons of the adult human cortex and cerebellum. These proteins (Mre11 and NBS1) were substantially reduced in the neurons of AD cortex. This finding suggests that the loss of the Mre11 complex may be associated with the pathogenesis of AD (27).

The Cdc25 phosphatases play key roles in cell cycle progression by activating cyclin-dependent kinases. The latter are absent from neurons that are terminally differentiated in adult brain. However, accumulation of mitotic phosphoepitopes, and re-expression and activation of the M phase regulator, Cdc2/cyclin B, have been described in neurons undergoing degeneration in AD. The structural hallmarks of AD neurodegeneration—neurofibrillary tangles and neuritic plaques—were prominently immunolabeled with Cdc25A antibodies. In addition, numerous neurons without visible structural alterations were also intensely stained, whereas control brain was very weakly positive (16).

Double-strand break repair requires DNA-dependent protein kinase complex, composed of a catalytic subunit, DNA-PKcs, and a regulatory component, Ku. Ku DNA binding activity was reduced in extracts of postmortem AD midfrontal cortex, but was not significantly different from the age-matched controls. Decreased Ku DNA binding correlated with reduced protein levels of Ku subunits, DNA-PKcs, and poly(ADP-ribose)polymerase-1. Expression of the base excision repair enzyme Ref-1, however, was significantly increased in AD extracts compared to controls. Ku DNA binding and DNA-PK protein levels in the AD cases correlated significantly with synaptophysin immunoreactivity, which is a measure of synaptic loss, a major correlate of cognitive deficits in AD (13).

Mammalian cells use several mechanisms to repair DNA damage generated by various insults. The predominant mechanism to repair double strand breaks is nonhomologous end joining (NHEJ), which utilizes the DNA-dependent protein kinase (DNA-PK) complex. When a cell-free DNA end joining assay was employed to determine whether NHEJ was reduced in nuclear cortical extracts from brains of AD versus normal subjects, end joining activity and protein levels of DNA-PK catalytic subunit were significantly lower in AD brains compared to normal controls. The amount of end joining activity was correlated with the expression of DNA-PK and was dependent on DNA-PK catalytic activity. This indi-

cates that repair of DNA double-strand breaks by the DNA-PK-dependent NHEJ pathway may be deficient in AD (54).

Spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) is a neurodegenerative disease that results from mutation of tyrosyl phosphodiesterase 1 (TDP1). The clinical phenotype of SCAN1 is associated with progressive degeneration of postmitotic neurons. El Khamisy *et al*. (17a) showed that in human cells, TDP1 is required for repair of chromosomal single-strand breaks arising independently of DNA replication from abortive top1 activity or oxidative stress. TDP1 is sequestered into multiprotein single-strand break repair (SSBR) complexes by direct interaction with DNA ligase IIIa, and these complexes are catalytically inactive in SCAN1 cells. These data identify a defect in SSBR in a neurodegenerative disease, and implicate this process in the maintenance of genetic integrity in postmitotic neurons.

In addition to slow protracted neurodegenerative disease, neuronal atrophy induced by acute trauma is also associated with accumulation of DNA damage. Support for this notion came from a study showing that motor neuron apoptosis in the adult spinal cord is controlled by upstream mechanisms involving DNA damage and activation of p53, and downstream mechanisms involving upregulated Bax and cytochrome *c* translocation, and activation of caspase-3. It was concluded that adult motor neuron death after nerve avulsion is DNA damage-induced, p53-and Bax-dependent apoptosis (41).

THE ROLE OF ABORTIVE CELL CYCLE IN NEURONAL DEGENERATION

Recent evidence suggests that abortive re-entry into the cell cycle represents an additional key mechanism of neuronal apoptosis. Reactivation of components of the cell cycle is often observed in dying neurons and may contribute to neuronal apoptosis in the developing brain and to neuronal degeneration in the adult brain (reviewed in Refs. 6 and 35).

Kruman *et al*. (36) postulated that cell cycle activation is a critical component of the DNA damage response in postmitotic neurons. When terminally differentiated cortical neurons were exposed to a genotoxic compound as well as to apoptotic inducers that are not genotoxic, the genotoxic compounds led to apoptosis accompanied by cell cycle reentry, whereas apoptosis initiated by stimuli that do not target DNA did not initiate cell cycle activation. Interestingly, Atm suppression attenuated both apoptosis and cell cycle reentry triggered by DNA damage but did not change the fate of neurons exposed to nongenotoxic compounds. These data suggest that cell cycle activation is a critical element of the DNA damage response of postmitotic neurons leading to apoptosis (36).

OXIDATIVE STRESS, DNA DAMAGE, AND NEURODEGENERATIVE DISEASES

Oxidative stress occurs when the production of reactive oxygen species (ROS), a normal product of cellular metabolism, is greater than the ability of the cell to repair the resulting damage. For that purpose, cellular oxygen concentrations are maintained with a narrow "nomoxic" range to circumvent the risk of oxidative damage from excess O_2 (hyperoxia) and of metabolic demise from insufficient O_2 (hypoxia) (52). pO_2 ranges from 90 to below 3 Torr in mammalian organs under normoxic conditions with arterial pO2 of 100 Torr or ~14% (48). Thus, "normoxia" for cells is an adjustable variable that is dependent on the specific localization of the cell in organs and the functional status of the specific tissue. The fact that oxygen is dangerous to the very life forms for which it has become an essential component of energy production is referred to as the "Oxygen Paradox." The first defense against oxygen toxicity is the sharp gradient of oxygen tension, seen in all mammals, from environmental level of 20% to tissue concentration of only 0.5–5% oxygen. These relatively low tissue levels of oxygen prevent most oxidative damage from ever occurring. Removal of this line of defense from cultured cells, that, under normal culturing procedures are exposed to conditions of O_2-rich room air (20% O_2), can result in the activation of specific O_2-sensitive signal transduction pathways that alter cellular phenotype and function. It has been reported that cardiac fibroblasts isolated from adult murine ventricle, cultured in 10% or 21% O_2 (high O_2, relative to the pO_2 to which they are adjusted *in vivo*), compared with 3% O_2, exhibit reversible growth inhibition and a phenotype indicative of differentiation (49). The role of abortive redox state in genome instability disorders and its effects on DNA damage response were described in detail in our previous reviews (4, 5).

As mentioned earlier, it has been estimated that around 2×10^4 DNA damaging events occur in every cell of the human body every day (2). A significant portion of the damage is caused by ROS. As early as 1952, Conger and Fairchild (10) demonstrated that increased oxygen pressure could lead to the accumulation of chromosomal aberrations. The effect of excessive production of ROS, and/or the inadequacy of the antioxidant cellular defense systems to neutralize them, are commonly referred to as oxidative stress.

Oxidation-reduction (redox) based regulation of gene expression appears to be a fundamental regulatory mechanism in cell biology. In contrast to the conventional idea that reactive species mostly serve as a trigger for oxidative damage of biological structures, we now know that low, physiologically relevant concentrations of ROS can regulate a variety of key molecular mechanisms (53). Thus, maintaining homeostatic levels of oxygen is critically important for proper neuronal functioning. One common product of nucleic acid damage by oxidation is 8-hydroxyguanosine (8OHG). Indeed, 8OHG immunoreactivity is widely used as a marker for evaluating the effect of oxidative stress on nucleic acids. This molecule can be induced by various environmental factors and is known to permanently damage cytoplasmic RNA and mitochondrial DNA, thereby contributing to neurodegeneration.

DNA damage cannot always be repaired properly, a situation that can precipitate cell death. An example is Cockayne syndrome that is caused by mutations in the CSA, CSB, or the xeroderma pigmentosum genes (XPG). The transcription-coupled repair pathway that involves the XPG and CSB proteins repairs 8OHG lesions and defects in this pathway are the cause of this syndrome (11, 38, 60). Another example of

DNA damage and how the lack of repair induces cell death is found in amyotrophic lateral sclerosis (ALS) (32, 57). Damaged DNA accumulates in familial ALS and 8OHG lesions increase in the motor cortex of patients with the sporadic form of the disease (8, 18). Since the accumulation of DNA damage is a signal for apoptosis, such damage may be partially responsible for the death of upper and lower motor neurons in ALS. Additional mechanisms of neurodegeneration, such as mitochondrial dysfunction leading to an increase in free radical generation, may also be involved in sporadic forms of the disease (18).

What are the cellular mechanisms that mediate oxidative insult-induced death? The upregulation of p53 in response to a diverse array of cellular insults ranging from ischemia/hypoxia and excitotoxicity to oxidative stress in multiple neuronal populations points to p53 as a key factor in neuronal death in response to different forms of acute insults and chronic neurodegenerative conditions. How these divergent cellular insults activate p53 in neurons is poorly understood, but oxidative DNA damage may be a major common feature integrating the different stress signaling pathways and initiating p53-mediated apoptosis (reviewed in Ref. 12). In addition to neurological disorders caused by acute insults, progressive neuronal death associated with enhanced p53 levels has been detected in chronic neurodegenerative diseases, including Parkinson's disease (PD) and AD. PD affects primarily dopaminergic neurons of the substantia nigra pars compacta. The mechanisms behind selected dopaminergic death in PD are unclear, but may involve oxidative stress that induces DNA damage and activation of p53 (7, 28). In AD, accumulation of the neurotoxic amyloid-β protein (Aβ) in the brain tissue is believed to be a major cause of the progressive degeneration and death of neurons (42). In the brain tissue of AD patients, damaged neurons exhibit increased p53 immunoreactivity (14), and a similar pattern of enhanced p53 levels was observed in the brain of transgenic mice overexpressing $A\beta^{1-42}$ (37). Interestingly, Uberti *et al*. (61) found that fibroblasts from AD patients were more resistant than those from control subjects to hydrogen peroxide treatment, although the extent of DNA damage induced by the oxidative injury was similar in both experimental groups. The protective mechanism of AD fibroblasts was related to an impairment of hydrogen peroxide-induced cell cycle arrest, and characterized by an accelerated re-entry into the cell cycle and diminished induction of apoptosis. Fibroblasts from AD patients also have profound impairment of hydrogen peroxide-activated p53 dependent pathway, which results in a lack of activation of p53 or p53-target genes, including p21, GADD45, and bax (61). p53 was found to be present in synapses where its level and amount of phosphorylation were increased following exposure of the cells to the DNA-damaging agent etoposide. The elevation of synaptic p53 levels was transcription independent, suggesting that such a local action of p53 may contribute to the dysfunction and degeneration of synapses that occur in various neurodegenerative disorders (24).

Mutations in the CSB gene cause Cockayne syndrome, a rare inherited disorder characterized by UV-sensitive severe neurodevelopmental and progeroid symptoms. CSB functions in the transcription-coupled repair (TCR) subpathway of nucleotide excision repair (NER) responsible for the removal of UV-induced and other helix-distorting lesions from the transcribed strand of active genes. A role for TCR in resistance to oxidative damage was established (15). PARP1 is a nuclear protein that protects the integrity of the genome by responding to oxidative DNA damage and facilitating DNA repair. PARP1 binds to single-strand DNA breaks, which activate the catalytic ability of PARP1 to add polymers of ADP-ribose to various proteins. It was found that CSB is present at the site of activated PARP1 after oxidative stress, identifying CSB as a new substrate of PARP1 and demonstrating that poly-ADP ribosylation of CSB inhibits its DNA-dependent ATPas activity (59).

CONCLUSIONS

Proper DNA damage response is critically important for appropriate functioning of every cell in our body, especially for neurons, which are long-living, highly active postmitotic cells. The DNA damage response is involved in every stage of the neuronal cell—from birth to death. The DNA damage response affects many cellular processes such as cell cycle arrest, activation of the DNA repair machinery, cellular metabolism, and the activation of various death programs. Thus, understanding the molecular mechanisms of the DNA damage response in neuronal cells is critically important for designing new strategies to treat such human diseases as genomic instability disorders and age-related neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

ABBREVIATIONS

A-T, ataxia telangiectasia; A-TLD, A-T like disorder; ATM, A-T mutated; Cdc, cell cycle serine/threonine protein phosphatase; CDK, cyclin dependent kinase; CNS, central nervous system; DAR, differentiation-associated repair; DNA, PK-DNA dependent protein kinase; DSBs, double-strand DNA breaks; GADD45, growth arrest and DMA damage 45; HR, homologous recombination DNA repair; 8OHG, 8-hydroxy guanine; Mre11, meiotic recombination 11; NBS, Nijmegen breakage syndrome; NER, nucleotide excision repair; NHEJ, non homologous end joint DNA repair; PARP, poly(ADP-ribose) polymerase; SCID, severe combined immunodeficiency; TCR, transcription-coupled repair; TS, transcribed strand; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling; XRCC4; Xray repair cross complementing protein.

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