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Mechanistic Insight into DNA Damage and Repair in Ischemic Stroke: Exploiting the Base Excision Repair Pathway as a Model of Neuroprotection

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Abstract

Stroke is a common cause of death and serious long-term adult disability. Oxidative DNA damage is a severe consequence of oxidative stress associated with ischemic stroke. The accumulation of DNA lesions, including oxidative base modifications and strand breaks, triggers cell death in neurons and other vulnerable cell populations in the ischemic brain. DNA repair systems, particularly base excision repair, are endogenous defense mechanisms that combat oxidative DNA damage. The capacity for DNA repair may affect the susceptibility of neurons to ischemic stress and influence the pathological outcome of stroke. This article reviews the accumulated understanding of molecular pathways by which oxidative DNA damage is triggered and repaired in ischemic cells, and the potential impact of these pathways on ischemic neuronal cell death/survival. Genetic or pharmacological strategies that target the signaling molecules in DNA repair responses are promising for potential clinically effective treatment. Further understanding of mechanisms for oxidative DNA damage and its repair processes may lead to new avenues for stroke management. *Antioxid. Redox Signal.* 14, 1905–1918.

Introduction

Stroke is one of the leading causes of mortality and morbidity, with enormous financial repercussions on health systems worldwide. Accumulating evidence suggests that cerebral ischemia-induced DNA damage plays a critical role in neuronal cell death. Endogenous oxidative DNA damage, in the forms of base damage and strand breaks, can be detected in the ischemic brain during stages preceding the manifestations of cell death, and is believed to trigger cell death *via* various intracellular signaling pathways. DNA repair systems, particularly base excision repair (BER), are endogenous defense mechanisms to combat oxidative DNA damage. Inhibiting DNA damage signals or enhancing DNA repair activity can serve as neuroprotective strategies against cerebral ischemic injury.

DNA Damage in Ischemic Stroke

Active versus passive DNA damage after ischemic brain injury

Emerging studies have demonstrated that DNA damage occurs in response to cerebral ischemia. According to the mechanisms of action, DNA damage can be classified into two distinctive types: active DNA damage and passive DNA damage.

Active DNA damage. Active DNA damage is mediated by DNA endonucleases, and is also referred to as endonuclease-mediated DNA damage. The best-studied active DNA damage is apoptotic DNA fragmentation, which is characterized by DNA double-strand breaks (DSBs). This fragmentation involves a cascade of cellular self-destruction, and usually occurs irreversibly at the late stage of cell injury. Two endonucleases, caspase-activated deoxynuclease (CAD) and apoptosis-inducing factor (AIF), have been implicated as the main endonucleases in the process of DNA fragmentation (Fig. 1).

Caspase-activated deoxynuclease. CAD is activated after caspase (3 or 7)-dependent degradation of its inhibitor, ICAD. Typically, CAD mediates the fragmentation of DNA at internucleosomal linker sites, giving rise to characteristic bands of 180–200 bp multiples in a ladder-like pattern on DNA gels. Several studies have shown that CAD is activated in vulnerable regions in the brain after transient global or focal

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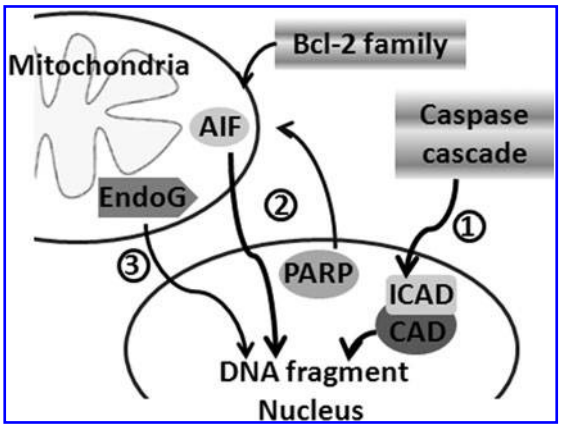

FIG. 1. Endonuclease-mediated active DNA damage. Active DNA damage involves a series of DNA endonucleases, including CAD, AIF, and EndoG. [1] Caspase cascades degrade ICAD, resulting in activation of CAD and subsequently DNA fragmentation. [2] AIF redistributes from mitochondria to nucleus upon activation through two upstream pathways: Bcl-2-family proteins and PARP-1. [3] EndoG translocates into the nucleus to cause DNA fragmentation and ultimately cell death. AIF, apoptosis-inducing factor; CAD, caspase-activated deoxynuclease; EndoG, endonuclease G; ICAD, inhibitor CAD; PARP, poly(ADP-ribose) polymerase.

ischemia and appears to be responsible for internucleosomal DNA fragmentation. However, DNA fragmentation after ischemia is far more complex than classic apoptosis. For instance, the induction of high-molecular-weight (HMW) DNA fragmentation has been reported to precede that of internucleosomal DNA fragmentation after ischemia (56). Thus, CAD may not be responsible for all types of DNA fragmentation in neurons after ischemia.

Apoptosis-inducing factor. AIF is a mitochondrial flavoprotein that translocates into the nucleus upon apoptotic disruption of mitochondrial membrane permeability and leads to large-scale DNA fragmentation. AIF is capable of producing HMW DNA fragments in response to cell death signals, including cerebral ischemia. AIF has been observed to translocate from mitochondria to the nucleus in neurons after transient cerebral ischemia, and this appears to be correlated with the selective vulnerability of neurons to ischemic insult (19, 61). AIF has been widely accepted to function in a caspase-independent manner. However, recent findings suggest that at least two pathways operate upstream of AIF release: one that depends on upstream Bcl-2-family proteins (*e.g.*, Bax) and caspases, and another that depends on poly(ADP-ribose) polymerase-1 (PARP-1) (17).

Others. The involvement of other endonucleases such as endonuclease G (EndoG) has also been documented in neuronal death after ischemic injury (Fig. 1). Similar to AIF, EndoG is a mitochondrial enzyme that translocates into the nucleus to cause cell death. It was proposed that AIF and EndoG may work together to induce DNA fragmentation after ischemia.

Taken together, the current evidence suggests a complex and possibly unique process involving more than one endonuclease that mediates ischemia-induced DNA fragmentation.

Passive DNA damage. Passive DNA damage is an endonuclease-independent process resulting from attacks by reactive oxygen species (ROS), and thus is referred to as oxidative DNA damage. Both direct and indirect mechanisms are involved in ROS-induced passive DNA damage.

Direct DNA attacks by ROS. ROS, mainly hydroxyl radicals, can directly attack DNA by extracting hydrogen atoms from deoxyribose and ribose, or adding protons to double bonds. Passive DNA damage consists of various DNA lesions, including base modifications, such as 8-oxodeoxyguanine (8-oxo-dG), 8-oxodeoxyadenine (8-oxo-dA), thymine glycol and thymidine glycol, 8-hydroxy-2′-deoxyguanosine (8-OHdG), 5-hydroxy-2′-deoxycytidine (5-OhdC), 5-hydroxy-2′-deoxyuridine (5-OhdU), and dihydrothymine. Common oxidative DNA lesions also include apurinic/apyridinic abasic (AP) sites, single-strand breaks (SSBs), DNA intra- or interstrand crosslinks, DNA–protein crosslinks, and damage to the deoxyribose moiety (14) (Fig. 2).

Indirect DNA attacks by ROS. The reaction of ROS with lipids or proteins will generate secondary reactive species and cause DNA damage indirectly. Peroxidation of polyunsaturated lipids produces DNA-damaging substances. For instance, lipid hydroperoxide-derived bifunctional electrophiles can covalently modify DNA (82). Exposure of amino acids, peptides, and proteins to radicals in the presence of O_2 generates hydroperoxides. These hydroperoxides are stable without exogenous catalysts (*e.g.*, heat, light, or redox-active transition metal ions), but decompose rapidly in the presence of these agents to generate a variety of radicals, including alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$). These radicals are shown to react with DNA to give DNA–protein cross-links and SSBs (55).

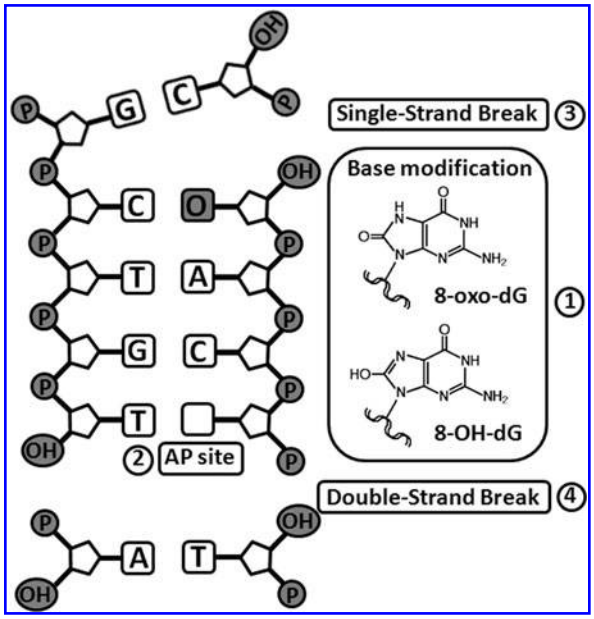

FIG. 2. Common oxidative DNA lesions in ischemic injury. [1] Base modifications such as 8-oxo-dG and 8-OHdG; [2] AP sites; [3] Single-strand break. [4] Double-strand break. 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxo-dG, 8-oxodeoxyguanine; AP site, apurinic/apyridinic abasic site.

In contrast to endonuclease-mediated active DNA damage, oxidative DNA damage is featured by its early onset. Oxidative DNA damage may occur as early as minutes after transient cerebral ischemia, whereas active DNA damage is still not detectable. Multiple types of oxidative DNA damage, including 8-oxo-dG, AP sites, and SSBs, have been observed in the ischemic brain either at the beginning or early in the reperfusion phase (14, 47). The greatest increase in oxidative DNA damage occurred during reperfusion, reflecting the spike of free radical generation during reoxygenation. Another important distinctive feature of oxidative DNA damage is that it is at least partially reversible. Postischemic oxidative DNA damage can be repaired in brain cells that eventually survive ischemia (14, 47); otherwise, it accumulates in cells and ultimately triggers cell death. Due to its early onset and reversibility, ischemia-induced oxidative DNA damage and its repair mechanisms, which are the focus of the current review, are of great interest for basic research and highly valuable for clinical therapies.

Oxidative DNA damage injures different cell types in the ischemic brain

Oxidative DNA damage accumulation is an important detrimental factor in the fate of ischemic brain cells. Although neuronal oxidative damage has been the focus of research for decades, it is becoming clearer that ischemic DNA damage affects not only neurons but also the whole network of brain cells (astrocytes, microglia, vascular cells *etc*.).

Neurons. Several lines of evidence indicate that DNA damage resulting from ischemia can decrease neuronal viability. First, ischemia and reperfusion are usually accompanied by DNA lesions that are found to be potent inducers of cell death. AP sites and SSBs, among the most prevalent oxidative lesions formed in neurons after ischemia, represent the type of DNA lesions that have potent cell-killing effects. Upon accumulation, AP sites prevent the process of DNA synthesis and gene transcription through the lesion sites and thus are directly lethal to cells. The accumulation of DNA SSBs may trigger cell death through several pro-death signaling pathways (51). Several studies have shown that AP sites and SSBs are accumulated in ischemic neurons that eventually develop DNA fragmentation and apoptotic cell death (14, 47), suggesting that oxidative DNA damage may be an important trigger of ischemic neuronal death. The presence of 8-oxo-dG, the most prevalent oxidative base modification, was also markedly increased in ischemic brain after reperfusion (47). 8-Oxo-dG has been associated with gene mutagenesis, giving rise predominantly to GC→TA transversions (48). Genes that are enriched in the formation of 8-oxo-dG may partially or completely lose their functional properties. Second, ionizing radiation (IR), ultraviolet irradiation, and DNA damage agents, including cytosine arabinoside and camptothecin, lead to neuronal apoptosis and/or necrosis, supporting a role for DNA modification in neuronal injury. Finally, loss of DNA repair exacerbates neuronal loss after ischemic stroke (discussed in detail in section III), indicating the importance of DNA integrity in neuronal viability.

Astrocytes and microglia. Oxidative DNA damage is also found in nonneuronal cells in *in vivo* ischemic models. 8-OHdG, a common form of oxidative DNA damage, was observed in both microglia and astrocytes in the ipsilateral striatum after focal cerebral ischemia (57). Both SSBs and DSBs were also identified in GFAP-positive astrocytes in the border zone of the infarct tissue, most evident at 72 h after reperfusion (14). Further, these astrocytes showed morphological changes consistent with apoptosis, suggesting that the accumulation of unrepaired DNA SSBs may contribute to ischemic astrocytic death, perhaps by triggering apoptosis. On the other hand, little is known about the destination of microglia after cerebral ischemia-induced oxidative damage. Further studies are warranted to elucidate the precise mechanisms responsible for DNA damage-induced microglia and astrocyte outcomes after ischemia.

Vascular cells. Vascular endothelial cells are also targets for oxidative DNA damage, although they are less vulnerable than neurons and astrocytes. Double staining of DNA polymerase I-mediated biotin-dATP nick-translation and vascular cell marker revealed that SSBs, although almost exclusively in neurons at earlier reperfusion periods, were also observed in endothelial cells in small blood vessel walls at later times (16–72 h of reperfusion) (14). The significance of elevated oxidative DNA damage in vascular cells has not been investigated.

Molecular signaling pathways that mediate oxidative DNA damage-triggered ischemic cell death

Ischemia-induced oxidative DNA damage is able to trigger a variety of pro-death signaling cascades, leading to ultimate cell death. The blockade of molecular events along these pathways has been demonstrated to offer neuroprotection against ischemic brain injury, emphasizing the necessity and importance for thorough investigation and understanding of this field.

PARP-1 overactivation and subsequent events

PARP-1 activation. PARP-1 is an abundant nuclear protein that is composed of three distinct domains: an N-terminal DNA-binding region able to recognize DNA strand breaks, an internal automodification region, and an nicotinamide adenine dinucleotide (NAD)-binding region that has all the catalytic activities of the full-length enzyme (38). PARP-1 activity remarkably increases in the presence of DNA strand breaks, especially SSBs. PARP-1 serves as a sensor to bind DNA breaks and activate downstream signaling events. Accumulating experimental data suggest that postischemic activation of PARP-1 occurs in practically every cell type of the affected brain region and significantly contributes to the extent of final damage.

Signaling pathways downstream of PARPs. The molecular mechanism underlying the detrimental role of PARP-1 in ischemic brain injury has been under active investigation (Fig. 3).

NAD^+ depletion. Upon binding to damaged DNA, PARP-1 forms homodimers and catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose, forming long branches of ADP-ribose polymers on target proteins such as histones and PARP-1 itself. Excessive DNA damage results in PARP-1 overactivation and subsequent depletion of NAD^+, driving the cells into cellular energetic depletion and mitochondrial disfunction (65). Depletion of cytosolic NAD^+ produces a block of neuron death independent of PARP-1 activation.

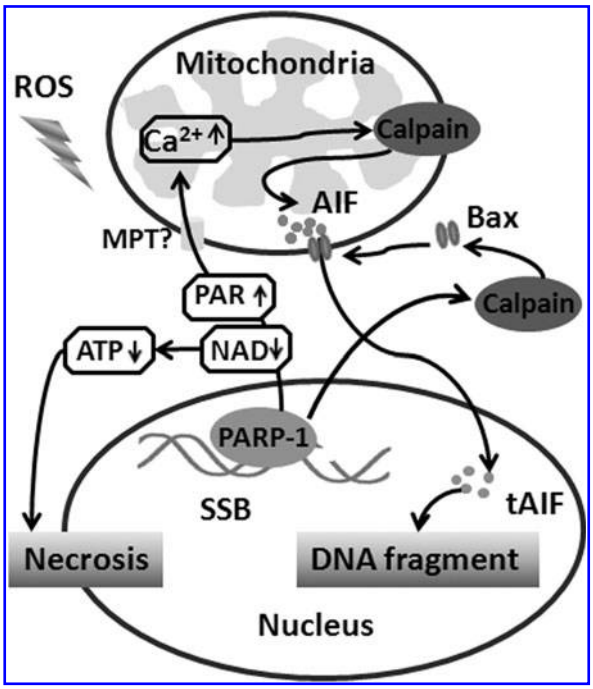

FIG. 3. PARP-1 overactivation and subsequent events. PARP-1 recognizes DNA breaks, especially SSB, and activates downstream signaling events. PARP-1 overactivation causes mitochondrial Ca^{2+} dysregulation, either by increased PAR polymer formation or NAD^+ depletion. Ca^{2+} dysregulation activates mitochondrial calpain, which truncates AIF. tAIF translocates to the nucleus where it causes DNA fragmentation and neuronal death. Activated calpain may also activate mitochondrial translocation of Bax and facilitate the release of AIF from mitochondria. MPT, mitochondrial permeability transition pore; NAD^+, nicotinamide adenine dinucleotide; PAR, poly(ADP-ribose); SSB, single-strand break; tAIF, truncated AIF.

Exogenous NAD^+ replenishment prevents neuronal death induced by PARP-1 activation upon DNA damage, suggesting NAD^+ depletion as a downstream event in PARP-1-mediated cell death (1). Of therapeutic interest, direct NAD^+ repletion reduces oxidative DNA damage and ischemic cell death in both *in vitro* cortical cultures (80) and an *in vivo* model of brain ischemia (87).

AIF release. More recently, the translocation of AIF from mitochondria to the nucleus was identified as a key step in the PARP-1 cell death process (15). PARP inhibition or PARP-1 knockout severely impairs the release of AIF from mitochondria. Likewise, activation of PARP or transfection of free polymer into susceptible cells leads to AIF-triggered apoptosis. The PARP-1-AIF association to neurons within the context of ischemic stroke has been revealed by specific PARP-1 inhibition, which attenuated the migration of AIF from mitochondria to the nucleus and protected neurons from death after oxygen–glucose deprivation *in vitro* and focal cerebral ischemia *in vivo* (6).

Ambiguity still exists with regard to the precise mechanism by which activation of PARP-1 could lead to AIF release. A causal link between NAD^+ depletion and AIF release has been suggested by studies showing that restoring NAD^+ levels blocks PARP-1-induced mitochondrial failure, AIF release, and cell death (1). However, many studies argue that PARP-1-induced AIF release may be independent of NAD^+ depletion. AIF release can be triggered by the action of poly(ADP-ribose) directly on mitochondria (89). Using an NMDA toxicity model, Vosler *et al.* further found that PARP-1 activation caused mitochondrial Ca^{2+} dysregulation, potentially *via* the mitochondrial permeability transition pore. Further Ca^{2+} dysregulation activates mitochondrial calpain, responsible for AIF truncation, and subsequent translocation to the nucleus (79). The precise mechanisms between PARP-1 activation and AIF release are probably more complicated than a single, straight pathway.

Others. Several distinct pathways, such as a sequential activation of PARP-1, calpains, and Bax (59), and a sequential activation of PARP-1, receptor-interacting protein 1, tumor necrosis factor receptor-associated factor 2, and c-Jun NH2-terminal kinase (83), have been proposed in nonneuronal cells. However, whether these pathways exist in neurons has not been elucidated. Further investigation is warranted.

Phosphatidylinositol 3-kinase-like kinase-associated pathways. Recruitment of DNA damage-associated phosphatidylinositol 3-kinase-like kinases (PIKKs) to DNA lesions are thought to be an important signaling event after DNA damage. The activation of PIKKs in response to DNA damage will control the activation and assembly of other enzymes and

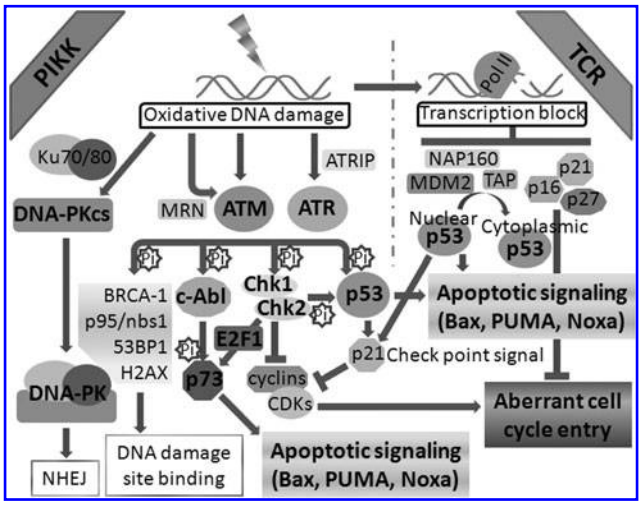

FIG. 4. PIKKs and TCR-associated pathways. The primary mechanism that has been identified for detection and downstream relaying of DNA damage involves the activation of PIKKs, primarily ATM, ATR, and DNA PKcs. DNA damage also results in significant alterations in DNA structure and inhibits RNA transcription, leading to the induction of TCR. Activation of ATM, ATR, and TCR may cause alterations of critical molecules, such as those involved in p53 nuclear export and cell cycle inhibition, potentially leading to apoptotic p53 signaling and/or aberrant entry in to the cell cycle. ATR and ATM may also activate apoptosis *via* p53-independent mechanisms. There is significant downstream crosstalk between all these signaling pathways, and whether the outcome is repair or cell death likely depends on both the extent and duration of the damage. ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad-3-related; DNA PKcs, DNA protein kinase catalytic subunit; PIKKs, phosphatidylinositol 3-kinase-like kinases; TCR, transcription-coupled repair.

proteins involved in DNA repair and/or pathways leading to cell death (Fig. 4).

PIKK activation. The main PIKKs activated in response to DNA damage include ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia and Rad-3-related (ATR), and DNA protein kinase catalytic subunit (DNA PKcs). Other PIKK members include mammalian target of rapamycin, suppressor of morphogenesis in genitalia-1, and transformation/transcription domain-associated protein.

Ataxia-telangiectasia mutated. ATM has kinase activity that can be activated directly by DNA damage, mainly DSBs. It can also be recruited to sites of damage by a complex of Mre11, Rad50, and Nbs1. Activation of ATM could phosphorylate various protein targets to activate cell cycle checkpoints and repair the DNA damage. If the damage cannot be repaired, ATM directs the cell to apoptosis.

Ataxia-telangiectasia and Rad-3-related. ATR is activated by SSBs and stalled DNA replication forks. Recruitment of ATR to SSBs and its activation requires ATR-interacting protein. Like ATM, ATR is important in DNA repair and the initiation of apoptotic signaling.

DNA–protein kinase catalytic subunit. DNA-PKcs is another PIKK that responds mainly to DSBs. DNA-PKcs is one molecule in a complex known as DNA-PK. The DNA-PK complex is required for the DNA repair of DSBs, which is accompanied by nonhomologous end-joining (NHEJ) (11). Upon IR-induced DNA damage, DSBs could be recognized by Ku70/80 heterodimer, another member in the DNA-PK complex. The binding of Ku70/80 with free ends of DSBs recruits DNA-PKcs and stimulates its kinase activity, allowing the formation of an active DNA-PK complex (49).

These three PIKKs—ATM, ATR, and DNA-PKcs—are all considered to be important in the sensing and repair of DSBs; however, DSBs have not yet been detected as an early event in cerebral ischemia. Nevertheless, experimental evidence indicates that PIKKs' activities change during ischemic brain injury and may contribute to neuronal death. Hypoxia and reoxygenation could result in activation of ATR (29) and ATM (30), respectively. DNA-PK components were also shown to be affected by ischemia and reperfusion to rabbit spinal cord. Ku DNA-binding activity is activated after reperfusion after short ischemic insult. Severe ischemic injury, however, was associated with decreased Ku DNA-binding activity and loss of DNA-PKcs, implying an exhausted DNA repair capacity in the presence of overwhelming of DNA damage (70). Although there is no direct evidence that PIKKs play a role in ischemic cell death, it has been reported that ischemic preconditioning (IP) leads to an increase in expression of Ku 70 in CA1 neurons, which was associated with resistance to ischemia-induced cell death (74).

Signaling pathways downstream of PIKKs. Activated PIKKs phosphorylate a wide variety of downstream targets, including c-Abl, breast cancer susceptibility gene (BRCA-1), p53, checkpoint kinase Chk1, Chk2, and H2AX. Activated PIKKs exert three crucial functions: regulating DSB repair, signaling cell-cycle checkpoints, and signaling apoptosis. There is significant cross-talk between these downstream signaling pathways, and whether the outcome is DNA repair or cell death likely depends on both extent and duration of the insults.

Apoptotic p53 signaling. The p53 protein is perhaps the most prominently noted molecule in studies of DNA damage and is sometimes regarded as the "universal sensor of genotoxic stress." ATM can directly phosphorylate p53 (Ser15) in response to IR, and ATR is critical in the same response to UV. P53 is also phosphorylated on Ser20 by Chk1 or Chk2, which are targets for ATR and ATM signaling (4). Phosphorylation of p53 on Ser15 or Ser20 stabilizes p53 against proteasomal degradation (18). Additionally, ATM-dependent pathways can indirectly contribute to p53 function by activating cyclin-dependent kinases (CDKs), phosphorylating murine double minute 2 (MDM2), a negative regulator of p53, or binding 14-3-3 proteins, which further enhances p53 DNA binding activity (18).

Increased p53 is seen in animal models after focal and global ischemia (77). Chemical inhibition of p53 expression can protect neurons growing *in vitro* from the effect of DNA-damaging agents, and can reduce the severity of brain injury after temporary focal ischemia in mice (20). Similarly, administration of pifithrin alpha, a specific inhibitor for p53 DNA binding activity, significantly improves outcomes after focal ischemia by reducing apoptosis (50). It also has been reported that brain injury was ameliorated in p53-deficient mice after permanent focal ischemia or transient global ischemia (88). The cell destructive effect of p53 could be attributed to the transcriptional activation of pro-apoptotic genes by p53, including upregulation of p53-upregulated modulator of apoptosis, Bax, and damage-regulated autophagy modulator; this mechanism is thought to lead to excitotoxic neuronal cell death in rat striatum (81). Mitochondrial dysfunction and cell death mediated by p53 have also been linked through regulation of SIVA, an apoptosis-selective p53 target gene whose expression in the brain is induced not only by DNA damage but also by focal ischemia (37). By transcription-independent mechanism, p53 was shown to be translocated to mitochondria after transient global ischemia, resulting in cytochrome c release and delayed neuronal cell death in the hippocampal CA1 sector (21). Although in nonneuronal cells, p53 has been shown to reduce apoptosis by transactivation of p21 and subsequent blockade of aberrant cell cycle entry (33), there is no evidence showing protective effect of p53 in postmitotic neuronal cells. Thus, the current studies provide abundant evidence that p53 mediates neuronal death induced by ischemia injury.

Cell cycle protein-related signaling pathways. A number of molecules that are targeted for phosphorylation by the PIKKs, including Chk1, Chk2, and p53, contribute to checkpoint signaling during cell division (25, 72). Initially, it appeared that such cell cycle molecules are not likely to play a significant role in postmitotic neurons. However, evidence exists that neuronal apoptosis may, in some cases, be due to aberrant re-entry into cell division. It has been reported that ischemia leads to expression and/or activation of cell cycle proteins such as cyclin D, CDK3, CDK5, p21, p25, and E2F1 (5). The activation of these cell proliferation proteins in neurons is a signal for death rather than cell division. A causative role for cell cycle proteins in activating death pathways in ischemic neurons is supported by the observations that

pharmacological inhibitors of CDKs are neuroprotective against *in vivo* ischemia (64), whereas overexpression of cell-cycle activity-inducing proteins such as E2F1 can cause neuronal death (43). Another study documented that neurons expressing $p16^{INK4a}$, an endogenous CDK inhibitor, tended to survive ischemic insult, whereas those losing expression of $p16^{INK4a}$ died upon ischemia (46). Similarly, hippocampal neurons expressing increased levels of p21, a potent and universal inhibitor of CDKs, are protected from ischemic injury (76). These findings suggest the involvement of aberrantly activated cell cycle proteins in ischemic apoptosis, which could be inhibited by the ATR/ATM-induced activation of Chk1/Chk2.

Despite the effort of ATM/ATR to halt aberrant cell cycle entry, thereby protecting cells against oxidative DNA damage, excessive DNA damage that overwhelms the repair capacity seems to redirect ATR/ATM to activate apoptosis. ATM/Chk2 can phosphorylate p53 at serine-20 and possibly at other residues (18). The activation of p53, in turn, mediates apoptosis. ATM- and/or ATR-activated ChK1 and ChK2 can also trigger p53-independent DNA damage-induced apoptosis *via* E2F1 activation. ChK1/ChK2 phosphorylates and activates E2F1 upon DNA damage induced by etoposide or camptothecin. E2F1 in turn stimulates transcription of the p73 gene, giving rise to an increased level of p73 protein, a homolog of p53. The p73 protein can either act as a cofactor of p53 or being pro-for p53-dependent apoptosis in response to DNA damage; or induce apoptotic even in the absence of p53. Apoptosis induced by p73 was shown to be mediated by transcriptional upregulation of p53-upregulated modulator of apoptosis, which in turn provokes Bax mitochondrial translocation and cytochrome *c* release. In addition, p73 was shown to activate the target gene that encodes NOXA, which causes mitochondrial dysfunction (23). The importance of E2F1 in ischemia-induced neuronal injury has been revealed by the observation that E2F1-null neurons or mice are more resistant to *in vitro* and *in vivo* ischemic injury, respectively (27). Further studies are needed to validate the molecular events downstream of E2F1 in ischemic models.

Others. ATM and ATR also act as kinases on several other downstream targets, including c-Abl, BRCA-1, and H2AX, which are involved in response to DNA damage. The c-Abl protein is a tyrosine kinase that can activate p73 and can transactivate other downstream apoptotic effectors, leading to p53-independent apoptosis. The importance of c-Abl in DNA damage signaling is shown by experiments demonstrating that apoptosis after treatment with IR is blocked in cells lacking c-Abl (69). BRCA-1 is a required cofactor for ATM/ATR-catalyzed phosphorylation of several targets, including p53 and Chk2 (24). After phosphorylation by ATM or DNA-PK, H2AX recruits repair proteins to the sites of DNA damage, including BRCA-1, p95/nbs1, and 53BP1. A phosphorylated form of H2AX has been observed concurrently with the initial appearance of HMW DNA fragments, and has been shown to be essential for CAD-mediated nucleosomal DNA fragmentation (71). Elevated activation of H2AX has been reported under ischemic stress (16), whereas its contribution to ischemic cell death has yet to be characterized.

Transcription-coupled repair-associated pro-death signaling pathway. Transcription-coupled repair is one of the elaborate arrays of DNA repair systems that have evolved in the cell to maintain genetic integrity. RNA polymerase II (Pol II) is a strong candidate for sensing DNA damage due to its role in genomic DNA transcription and its constant surveillance of the genome. The stalled RNA Pol II complex seems to be the damage-recognition signal in transcription-coupled repair and attracts DNA repair machinery (2). The DNA lesions encountered by Pol II may result in cell death when they are unrepairable, either due to loss of proteins critical to survival or *via* p53-mediated apoptosis signaling. One possible mechanism by which p53 is elevated after transcription blockade involves MDM2. MDM2, a negative regulator of p53, binds within the p53 N-terminal transactivation domain and enhances p53 nuclear export, ubiquitination, and degradation. MDM2 levels decrease after transcription blockade, resulting in nuclear accumulation of p53 and subsequent cell death (54). Another mechanism for p53 accumulation is related to altered mRNA from the nucleus, which blocks the export of nuclear-export signal-containing proteins, including p53 (62).

DNA damage as a biomarker for stroke

Several oxidative DNA lesions, such as 8-oxo-dG, 8-OHdG, and also some DNA repair enzymes, such as 8-oxoguanine DNA glycosylase (OGG1), have been proposed as biomarkers for many pathological conditions. Since these DNA lesions have also been demonstrated to increase in the ischemic brain, it is likely that these biomarkers can also indicate the oxidative stress induced by stroke.

One important characteristic of biomarkers is their accessibility *via* blood or urine, two of the common routes of biochemical examinations in clinical scenarios. Base modifications, one type of direct marker of DNA damage, are thought to localize in the nucleus. However, it is understood that, after excision from DNA, the oxidatively modified lesions are released into the bloodstream and consequently appear in the urine. Thus, effort has been exerted to identify biomarkers for the oxidative stress induced by stroke. It is also important to build up the time line profile for the candidates of stroke biomarkers to be detectable in blood or urine.

8-Hydroxy-2′-deoxyguanosine. Formation of 8-OHdG is regarded as a hallmark of oxidative DNA damage in nuclear DNA extracts. Increased immunoreactivity of 8-OHdG has been shown in the gerbil hippocampal CA1 region after transient focal ischemia (35). It has been widely used as a marker of oxidative DNA damage in cerebral ischemic models (41, 61). Interestingly, a recent study demonstrates that urinary 8-OHdG is an excellent marker for oxidative DNA damage (84), indicating that urinary 8-OHdG may be a promising candidate for a biomarker of stroke-induced DNA damage.

8-Oxodeoxyguanine. There is a common belief that the presence of 8-oxo-dG in urine represents the primary repair product of oxidative DNA damage *in vivo*, and that this compound may reflect the involvement of the nucleotide excision repair (NER) pathway. However, the route by which 8-oxo-dG arises in extracellular matrices is still controversial (63).

8-Oxoguanine. Due to its poor solubility, the analysis of 8-oxoguanine (8-oxo-Gua) in urine has presented particular difficulties. However, it has been recently described that

8-oxo-Gua can be specifically recognized and removed by OGG1 in human cells. Therefore, urinary assays that measure 8-oxo-Gua reflect glycosylase activities (63).

Repair of DNA Damage in Stroke

DNA repair pathways in the brain

Given the massive number of oxidative DNA lesions and mutations occur in response to cerebral ischemia, strong repair mechanisms must take actions to remove these lesions and mutations, and thus help neurons to survive. Prompt and efficient DNA repair in response to oxidative injury is particularly important in the brain due to the limited self renewal capacity of neurons. The three major DNA repair mechanisms are as follows.

DNA excision repair

Base excision repair. BER is the primary DNA repair pathway with the ability to fix base lesions that arise due to oxidative damage, thus ensuring the integrity of the genome. In response to ischemic and reperfusion injury, oxidative DNA damage markedly increases and may subsequently induce neuronal cell death, resulting in a great demand for BER. Unveiling the mechanisms and regulations of BER activities in the ischemic brain, which will be discussed in detail in the following section, will implicate novel therapeutic targets of stroke.

Nucleotide excision repair. In mammalian cells, NER is the major repair pathway for the removal of bulky adducts induced by UV radiation or other environmental carcinogens. However, there is evidence that it may also act upon common, nonbulky, oxidative DNA damage such as 8-oxo-dG and thymine glycol. Lipid oxidation products produce malondialdehyde-deoxyguanosine adducts in DNA that block transcription. Therefore, NER may be of more importance in repairing oxidative damage than was thought previously.

The NER process involves the action of 20–30 proteins in successive steps: DNA damage recognition, assembly of a multiprotein complex at the damaged site, and removal of the damage-containing oligonucleotide between the two nicks. The resulting gap is filled by a DNA polymerase, and the newly synthesized strand is sealed by a DNA ligase. Among those involved in NER, excision repair cross-complementing 8 (ERCC8) and ERCC6 were observed to associate with the repair of oxidative DNA damage of 8-OHdG and 8,5′-cyclopurine-2′-deoxynucleosides (2). Another important protein, ERCC1, also noted as a rate-limiting component of NER, was demonstrated to be capable of protecting neurons against ischemic injury in the rat brain. Overexpression of ERCC1, by injection of expression plasmids, significantly reduced the accumulation of DNA-damaged neurons and ischemic injury. In contrast, reduced endogenous ERCC1 levels, produced by specific antisense, increased DNA damage accumulation and ischemic brain damage following middle cerebral artery occlusion (MCAO), a transient focal cerebral ischemia model (31).

Direct reversal of DNA damage. In this type of repair, the damaged base is repaired through removing only the alteration to the base but not the base itself. O_6-methylguanine-DNA methyltransferase (MGMT) is the major protein involved, and it removes alkyl groups from the O_6 position of guanine and to a lesser extent the O_4 position from thymine. MGMT is expressed in both neurons and glia in the brain. It was shown that astrocytes have more MGMT activity and are more proficient in the repair of O_6-meG adducts than oligodendrocytes or microglia. However, questions still remain as to whether and to what extent a deficiency in this type of repair in neurons contributes to neurotoxicity (22).

Nonhomogenous endpoint jointing pathway. Although the number of DSBs is very small as compared with SSBs, they can be lethal to cells. Therefore, brain cells must have some mechanism to repair this type of damage. DSBs can be repaired by either homologous recombination or NHEJ. In nondividing cells like neurons, NHEJ may constitute the sole machinery for repairing the DSBs (68). Although it appears to be an unlikely event, there is evidence to show that DSBs also occur by homologous recombination. Proteins including, but not limited to, Ku70, Ku80, DNA ligase IV, and X-ray cross-complementing group 4 (XRCC4) are part of the NHEJ repair pathway (22). Ku70 and Ku80 are reduced after transient focal cerebral ischemia in mice. Cells with reduced levels of Ku proteins also exhibit DNA fragmentation, indicating the importance of these proteins in the repair and survival of neuronal cells after ischemia (39). Upregulated Ku70 was observed 1–3 days after global cerebral ischemia after sublethal IP (74). In neonatal rats challenged by hypoxia-ischemia, Ku70 was found colocalized within cells that had undergone proliferation in the peri-infarct region at 8 weeks after the insult, indicating the association between Ku70 and the repair process after cerebral ischemic insults (58).

Molecular pathways of BER in the brain

Most, if not all, of the DNA damage induced by ROS in mammalian cells is repaired and, possibly, reversed back to normal *via* the BER pathway. BER is initiated by various DNA glycosylases, which excise altered bases by hydrolytic cleavage of the base-sugar bond, generating a regular AP site in double-stranded DNA. In mammalian cells, AP endonuclease 1 (APE1), the major APE, cleaves the phosphodiester backbone 5′ to the AP site, leaving a nick with a 3′-OH group and a 5′-deoxyribose phosphate (dRP) residue. The downstream steps of BER, including strand incision, gap-filling and ligation, are achieved through at least two distinct subpathways—short patch and long patch (75) (Fig. 5).

Short-patch BER pathway. The short-patch BER pathway refers to a process involving sequential single-nucleotide gap-filling steps. It repairs regular AP sites with unmodified dRP residues. The dRP residue is removed *via* β-elimination by the 5′-lyase activity of DNA polymerase β (Polβ), which is the rate-limiting step. Polβ also synthesizes a single nucleotide to fill the gap. The nick between the synthesized nucleotide and the DNA template is sealed by DNA ligase III-XRCC1 complex (75).

Long-patch BER pathway. In the case where certain AP sites are altered by ROS, the lyase activity of Polβ (β elimination) is inhibited, whereas the polymerase activity of Polβ still functions. These lesions are efficiently repaired *via* an alternative pathway, the so-called long-patch BER pathway, which produces a repair tract of at least two, but most frequently four, nucleotides. A short dRP-containing oligomer downstream of the AP site, but not the dRP group alone, is

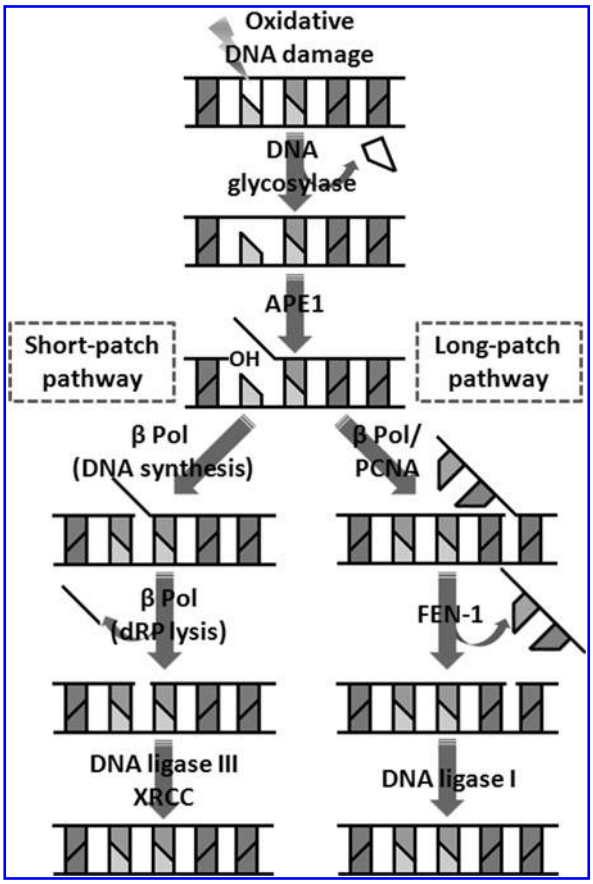

FIG. 5. BER pathway. BER is initiated by various DNA glycosylases, which excise altered bases by hydrolytic cleavage of the base-sugar bond, generating a regular AP site in double-stranded DNA. APE1 cleaves the phosphodiester backbone 5′ to the AP site, leaving a nick with a 3′-OH group and a dRP residue. In the short-patch pathway, DNA Polβ cleaves the dRP residue and synthesizes a single nucleotide to fill the gap. DNA ligase III-XRCC1 complex seals the nick between synthesized nucleotide and the DNA template. In the long-patch pathway, the structure-specific FEN1 cleaves the dRP-oligomer, Polβ/PCNA fills the gap, and DNA ligase I seals the nick. APE, AP endonuclease; BER, base excision repair; dRP, 5′-deoxyribose phosphate; DNA Polβ, DNA polymerase β; FEN1, flap endonuclease 1; PCNA, proliferating cell nuclear antigen; XRCC, X-ray cross-complementing group.

formed as the major excision product. The structure-specific flap endonuclease 1 (FEN1), which preferentially cleaves unannealed 5′ flap structures in DNA, is essential in the excision of the dRP-oligomer (67). The nucleotide gap at the lesion site is filled by strand displacement DNA synthesis *via* the action of Polβ, and the nick is subsequently sealed by DNA ligase I. FEN1 is known to stimulate Polβ-mediated DNA synthesis on a long-patch BER substrate, and Polβ is required for FEN1 to specify the sizes for excision, suggesting that these two proteins interact functionally and mechanistically (67).

The precise mechanism pertaining to the choice to proceed *via* the long-patch or short-patch BER for repairing oxidative lesions is not fully understood. One study suggested that the dRP intermediate produced by APE activity might be involved in the choice of BER subpathway. If the dRP can be efficiently removed by the dRP lyase activity of Polβ, BER proceeds by the short-patch mechanism; if not, the long-patch mechanism will be activated to avoid generating a nick that is refractory to the action of a DNA ligase. Another hypothesis suggests that the relative ATP concentration near the AP site is important for the switch from short-patch to long-patch BER (66). Long-patch BER tends to occur at low ATP concentrations, whereas short-patch BER appears to be the preferred mechanism with elevated ATP concentrations (66). Generally, the long-patch pathway is particularly significant in the repair of DNA damage induced by oxidative stress, since the oxidized AP site appears to be the prominent type of AP site accumulated in the ischemic brain (47).

Proteins interact with the BER pathway. Numerous proteins have been shown to interact with DNA glycosylases, FEN1, DNA polymerase, and other components of BER, and to ensure completion of the BER pathway (Fig. 6).

X-ray cross-complementing group 1. XRCC1 mediates BER by forming repair complexes with Polβ and DNA ligase III. It has been shown that XRCC1 stimulates the activity of DNA ligase III in the ligation step of BER (9). However, it seems that XRCC1 itself has no known enzymatic activity. It functions as a scaffold protein to coordinate short-patch BER (45).

Proliferating cell nuclear antigen. Proliferating cell nuclear antigen (PCNA) is an important, although not essential, component of the protein complex responsible for long-patch BER. PCNA strongly stimulates the 5′-flap cleavage activity of

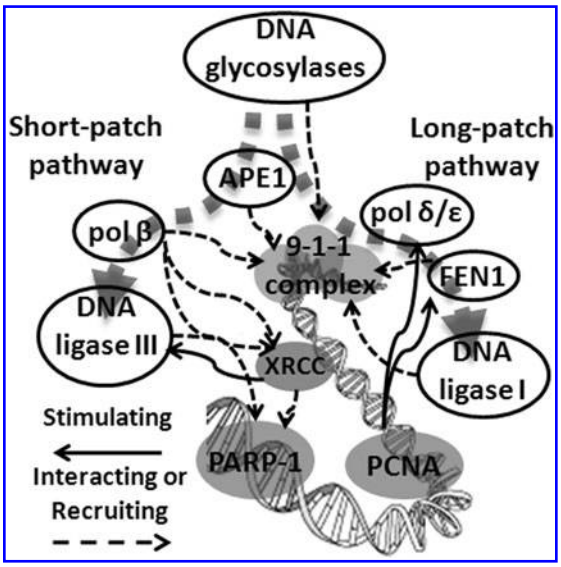

FIG. 6. Proteins interact with short-patch and long-patch BER pathways. XRCC1 functions as a scaffold protein to form repair complexes with Polβ and DNA ligase III, and stimulates the activity of DNA ligase III in the short-patch pathway. PCNA strongly stimulates the 5′-flap cleavage activity of FEN1 and DNA Polδ/ε in the long patch pathway. Rad9, Rad1, and Hus1 complex recognizes DNA damage and interacts with DNA glycosylases, APE1, Polβ, FEN1, and DNA ligase I. PARP-1 recognizes and also interacts with XRCC1 and Polβ. 9-1-1 complex, Rad9, Rad1, and Hus1 complex; DNA pol, DNA polymerase.

FEN1 (26). It is suggested that long-patch BER proceeds by either a PCNA-dependent pathway involving the use of DNA Polδ/ε or a PCNA-independent pathway that uses only DNA Polβ (42).

Rad9, Rad1, and Hus1 complex. The heterotrimeric protein complex composed of Rad9, Rad1, and Hus1 plays a significant role in the early recognition of DNA damage and recruit of appropriate proteins to the repair site. The Rad9, Rad1, and Hus1 complex interacts with several of the proteins involved in the proposed BER pathways, including DNA glycosylases, APE1, Polβ, FEN1, and DNA ligase I (3).

Poly(ADP-ribose) polymerase. Both the short-patch and long-patch BER pathways generate SSBs in DNA as repair intermediates, which could be recognized by the specific SSB binding domain of PARP. It has been proposed that PARP provides a nick-sensing function and subsequently recruits the BER complex to the nick through interaction with XRCC1. Recent evidence indicates that the interaction of PARP-1 with BER factors, such as XRCC1 and Polβ, can be regulated by p21 to promote efficient DNA repair (10).

The BER pathway is critical for cell survival after DNA damage

A strong linkage between cellular BER capacity and the fate of cells has been defined in nonneuronal mammalian cells that are subjected to oxidative stress or other genotoxic insults. At least three lines of evidence are available to support a critical role of BER activity in promoting cell survival after DNA damage.

Compromised BER sensitizes cells to DNA lesions. Several studies have demonstrated that defective repair of endogenous DNA lesions enhances vulnerability of cells to DNA damage in nonneuronal cells. Similar effects are seen in primary neuronal cultures as well, where downregulation of APE by siRNA leads to an increase of phosphorylation of H2AX, a DNA damage-associated molecule, and a reduction of cell viability after exposure to H_2O_2 (78). Also, suppression of uracil-DNA glycosylase by expression of an antisense construct induces apoptosis of primary hippocampal neurons (44).

Disruption of BER genes leads to defective neurogenesis. Targeted ablation of a BER gene, Polβ, or APE in mice results in embryonic death at an early or mid-gestation phase with an evident defect in neurogenesis (60). Polβ knockout mice exhibit a defective neurogenesis characterized by apoptosis in postmitotic neuronal cells, indicating that defective repair of endogenous DNA damage is destructive to neural development (60).

Enhanced BER promotes neuronal cell survival. A number of studies show that enhanced BER activity enables cells to become more resistant to oxidative DNA damage. For example, increasing APE expression in CHO cells by either sublethal ROS or gene transfection increased cell viability in response to DNA-damaging agents. Upregulation of Polβ activity also protected cells against DNA damage-induced cytotoxicity (34). The protective effect of enhanced BER in ischemic/reperfusion injury will be discussed in a later section.

Regulation of BER in the ischemic brain

Inducible BER response after sublethal ischemia. It is suggested that several components in the BER pathway are inducible in the brain in response to sublethal ischemic challenges. The first evidence came from a study showing that expression and activity of OGG1 were significantly increased in the mouse brain after 90 min of forebrain ischemia and 20–30 min of reperfusion (53). A later study showed dynamically the inducible responses of nuclear BER in the rat MCAO model of ischemia. According to this study, overall nuclear BER activity was markedly and persistently, up to 72 h, increased during reperfusion in the frontal/parietal cortex, the region surviving ischemia, due to the upregulation and activation of selective BER enzymes, particularly Polβ and OGG1 (47). More recent studies showed that preconditioning animals by three 10-min episodes or 30 min of MCAO significantly increased total BER activity and the BER proteins, including XRCC1, OGG1, DNA Polβ, APE, and DNA ligase III, after reperfusion (52). Focusing on APE, Xue *et al.* found that activation of the ERK signal transduction pathway increased expression of phospho-APE. This facilitated the repair of DNA damage, protecting neurons from apoptosis after cerebral ischemia and reperfusion (85). In addition to the induction of BER in nuclear extract, a similar inducible BER response was observed in brain mitochondria after brief focal ischemia in the rat (13).

Downregulated BER activity after a severe ischemic insult. Although induced in response to sublethal ischemia, BER activity is downregulated after a severe ischemic insult. In contrast to the regions that survived ischemia, where 8-oxo-dG and AP sites were efficiently repaired during reperfusion, DNA lesions were poorly repaired in the ischemic core destined to infarct. The failure of BER was due to the persistent decrease of multiple components of the BER pathway in both nucleus and mitochondria in this region, and may have contributed to cell death after ischemia (13, 47). Further evidence comes from the observations that expression of APE and the number of APE/Ref-1-positive cells were decreased after severe cerebral ischemia (61). Also, decreased expression of XRCC1 was found in all regions after severe cerebral ischemia, which may contribute, at least in part, to DNA fragmentation. A more recent study suggested that early induction of MMPs, an enzyme cleaving PARP-1 and XRCC1, in the nucleus after stroke, contributes to a reduction of DNA BER function (86).

Regulation of BER activity with aging. Numerous human and animal studies have demonstrated that oxidative nuclear DNA damage markedly increases with age in various tissues, including brain. It was estimated that the rate of 8-oxo-dG formation in brain tissue was accelerated in old mice compared with young mice (28). This study also found a threefold greater increase of 8-oxo-dG levels in mitochondrial DNA than in nuclear DNA, suggesting that mitochondrial DNA suffers greater damage with aging. Accumulating evidence strongly suggests that the age-dependent decline in BER activity may explain the oxidative DNA damage accumulation with age. For example, a significant decrease in DNA Polβ was seen in aging rat brain neurons. Young mice generally express 50% more DNA Polβ in response to oxidative stress

than old mice (7). An *in vivo* study in mice found that the decreased DNA Polβ activity contributes to the age-associated decline in BER in the brain (8). The BER decline may also be attributed to reduced glycosylase activity. A significant age-dependent decrease in incision activities of three glycosylases was observed in mitochondria of different brain regions, whereas variable patterns of change were seen in nuclei (36). Chen *et al.* found that decline of overall BER activity with age was attributed to the decreased expression of repair enzymes such as 8-OHdG glycosylase and DNA Polγ and, consequently, contributed to the age-dependent accumulation of oxidative DNA lesions in brain mitochondria (12). Further study on aging rat brain neurons found that both DNA Polβ and DNA ligase were needed to restore BER (68).

It is well known that stroke occurs mostly in elderly people. Patient outcomes after stroke are highly influenced by age. The age-dependent decline in BER activity may contribute to the increase in vulnerability to ischemic stroke in the elderly. Therefore, an attractive approach to limiting oxidative DNA damage and improving outcomes after ischemic stroke in the elderly would be to reverse the age-dependent decline of BER activity.

Enhancing the BER pathway as a potential neuroprotective strategy for ischemic neuronal injury

IP has been reported to enhance BER activity, contributing to IP-induced neuroprotection against cerebral ischemic injury (52). Induction of the BER proteins, including XRCC1, DNA Polβ, and DNA ligase III, was seen after reperfusion in ischemic preconditioned brain. Moreover, an increase in binding between XRCC1 and DNA Polβ was seen under these conditions, as might be expected during formation of functional BER complexes (52). Several pharmacological agents have been demonstrated to increase BER activity and protect against DNA damage-induced neuronal death after ischemia. Increased BER activity by NAD^+ repletion was reported to confer marked neuroprotection against ischemic cell death in rat primary neuronal cultures subjected to oxygen-glucose deprivation (80). Ebselen, a glutathione peroxidase mimic, was reported to attenuate DNA damage by enhancing repair activity, assessed by OGG1, in the thalamus after focal cortical infarction in hypertensive rats (32). Induction of APE activity was shown to be a unique strategy for neuroprotection against ischemic injury. Directly increasing APE/Ref-1 activity by administering an APE-mimicking peptide (40) enhanced DNA repair and promoted neuronal cell survival after transient focal cerebral ischemia. Most recently, intracerebral administration of pituitary adenylate cyclase-activating polypeptide, an endogenously occurring small neuropeptide, induced expression of APE1 in hippocampal neurons and markedly reduced oxidative DNA stress and hippocampal CA1 neuronal death after transient global ischemia (73).

The approach of enhancing BER activity by gene transfection was demonstrated recently. A study by Kim *et al.* found that intracerebral injection with Adv-APE, the adenoviral vector harboring the entire APE/Ref-1 gene-coding sequence, effectively inhibited the loss of APE after ischemia/reperfusion injury. It was then demonstrated that preischemic treatment of adenovirus-APE diminished the nuclear accumulation of AP site lesions and 8-OHdG, and significantly decreased TUNEL-positive cells and infarct volume after ischemia/reperfusion injury (41). Another study showed that ERCC1 overexpression, by injection of the expression plasmids into the rat brain, significantly reduced the accumulation of DNA-damaged neurons and ischemic cell death after MCAO (31). Given the relatively acute nature of cell death after stroke and the small time window of opportunity for stroke treatment, gene delivery, which needs relatively long time to take effect, may not be a legitimate therapeutic strategy for ischemic stroke. Nevertheless, gene delivery is a very useful technique to help elucidate molecular mechanisms for cell damage after stroke and identify critical therapeutic targets. Conceptually, small molecule-based or small peptide-based BER enhancers may hold greater promises for potential therapeutic intervention in stroke patients.

Summary and Future Directions

The past two decades have brought commendable research efforts and considerable advances in understanding the mechanisms for oxidative DNA damage-related cell apoptosis/necrosis. This article reviews molecular pathways by which oxidative DNA damage is triggered and repaired in cells, and their potential impact on ischemic brain injury. A growing body of evidence demonstrates various forms of oxidative DNA damage and emphasizes their importance in ischemic injury. Unrepaired oxidative DNA damage has been related to neuronal apoptosis and necrosis after cerebral ischemia. For the survival of neuronal cells, a fully functional DNA repair system is essential to combat oxidative DNA damage. In the brain, several repair mechanisms collaborate to maintain the integrity of the genome, among which the BER pathway predominates. Components in BER pathway have been shown to be inducible in response to sublethal ischemia and hypoxia. However, we are still apparently at early phases in the research on oxidative DNA damage in cerebral ischemia. Inconsistencies still exist in reports, likely due to the differences in strain or age of animals used. There are also numerous unanswered questions in this field. For example, it is not yet well characterized which cell death signaling cascades are specifically triggered by oxidative DNA damage in ischemic brain. Another consideration for the future is the role of oxidative DNA damage in nonneuronal cells, which, although reported, has not been well studied. It seems particularly promising that pharmacological and genetic strategies inducing BER activity are neuroprotective in neuronal cultures and in *in vivo* animal models of stroke. A more challenging question that needs to be addressed is how well these results can be translated into clinical scenarios. Also, it is intriguing to identify which forms of oxidative DNA damage could serve as clinically feasible biomarkers for ischemic brain injury. Further achievements in these areas will lead to new avenues for stroke management.

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Abbreviations Used

8-OHdG = 8-hydroxy-2′-deoxyguanosine
8-oxo-dG = 8-oxodeoxyguanine
8-oxo-Gua = 8-oxoguanine
9-1-1 complex = Rad9, Rad1, and Hus1 complex
AIF = apoptosis-inducing factor
AP sites = apurinic/apyridinic abasic sites
APE1 = AP endonuclease 1
ATM = ataxia-telangiectasia mutated
ATR = ataxia-telangiectasia and Rad-3-related
BER = base excision repair
CAD = caspase-activated deoxynuclease
CDKs = cyclin-dependent kinases
Chk = checkpoint kinase
DNA PKcs = DNA protein kinase catalytic subunit
dRP = 5′-deoxyribose phosphate
DSBs = double-strand breaks
EndoG = endonuclease G
ERCC = excision repair cross-complementing
FEN1 = flap endonuclease 1
HMW = high molecular weight
ICAD = inhibitor CAD
IP = ischemic preconditioning
MCAO = middle cerebral artery occlusion
MDM = murine double minute
MGMT = O_6-methylguanine-DNA methyltransferase
NAD = nicotinamide adenine dinucleotide
NER = nucleotide excision repair
NHEJ = nonhomologous end-joining
OGG1 = 8-oxoguanine DNA glycosylase
PAR = poly(ADP-ribose)
PARP-1 = poly(ADP-ribose) polymerase-1
PCNA = proliferating cell nuclear antigen
PIKKs = phosphatidylinositol 3-kinase-like kinases
Pol II = RNA polymerase II
Polβ = DNA polymerase β
ROS = reactive oxygen species
SSBs = single-strand breaks
tAIF = truncated AIF
TCR = transcription-coupled repair
XRCC1 = X-ray cross-complementing group 1

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