Apurinic/Apyrimidinic Endonuclease 1 Upregulation Reduces Oxidative DNA Damage and Protects Hippocampal Neurons from Ischemic Injury

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Abstract

Aims: Apurinic/aprimidinic endonuclease 1 (APE1) is a multifunctional enzyme that participates in base-excision repair of oxidative DNA damage and in the redox activation of transcription factors. We tested the hypothesis that APE1 upregulation protects neuronal structure and function against transient global cerebral ischemia (tGCI). **Results:** Upregulation of APE1 by low-dose proton irradiation (PI) or by transgene overexpression protected hippocampal CA1 neurons against tGCI-induced cell loss and reduced apurinic/aprimidinic sites and DNA fragmentation. Conversely, APE1 knockdown attenuated the protection afforded by PI and ischemic preconditioning. APE1 overexpression inhibited the DNA damage response, as evidenced by lower phospho-histone H2A and p53-upregulated modulator of apoptosis levels. APE1 overexpression also partially rescued dendritic spines and attenuated the decrease in field excitatory postsynaptic potentials in hippocampal CA1. Presynaptic and postsynaptic markers were reduced after tGCI, and this effect was blunted in APE1 transgenics. The Morris water maze test revealed that APE1 protected against learning and memory deficits for at least 27 days post-injury. Animals expressing DNA repair-disabled mutant APE1 (D210A) exhibited more DNA damage than wild-type controls and were not protected against tGCI-induced cell loss. **Innovation:** This is the first study that thoroughly characterizes structural and functional protection against ischemia after APE1 upregulation by measuring synaptic markers, electrophysiological function, and long-term neurological deficits *in vivo*. Furthermore, disabling the DNA repair activity of APE1 was found to abrogate its protective impact. **Conclusion:** APE1 upregulation, either endogenously or through transgene overexpression, protects DNA, neuronal structures, synaptic function, and behavioral output from ischemic injury. *Antioxid. Redox Signal.* 22, 135–148.

Introduction

DNA DAMAGE IS one of the most detrimental consequences of cerebral ischemia, and it can be categorized into two major forms: active and passive (17, 38). Active DNA damage is a part of a well-studied cascade of cellular suicide programs and is usually irreversible. This form of DNA damage is elicited by DNA endonucleases and involves double-strand DNA breaks during apoptosis. Passive DNA damage is also known as oxidative DNA damage and results from direct or indirect

attacks by reactive oxygen species during reperfusion (15, 38, 55). Passive DNA damage can consist of DNA-protein cross-links, apurinic/aprimidinic (AP) sites, single-strand breaks, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation (15, 17, 38). In contrast to active DNA damage, oxidative DNA damage occurs soon after ischemia, sometimes within minutes, and is at least partially reversible (17, 34). In order to reverse this oxidative damage, neurons have evolved base-excision repair (BER). BER is initiated by DNA glycosylases that excise bases through hydrolytic cleavage of the base-sugar bond,

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Innovation

The present study demonstrates that apurinic/apyrimidinic endonuclease 1 (APE1) mediates the mild stress-induced effects of ischemic preconditioning and proton irradiation. The present study also shows for the first time that APE1 upregulation elicits functional protection of the hippocampus against ischemic injury by partially preserving synaptic structures and their electrophysiological integrity. These findings connect DNA damage with the eventual collapse of synaptic communication. We also present the novel finding that spatial learning and memory deficits were reduced by APE1 transgene expression for the long term. Finally, in contrast to fully functional APE1, a DNA repair-defective mutant completely failed to provide any neuroprotection.

thereby generating temporary AP sites (11, 38). Left unattended, AP sites block DNA replication and transcription and promote double-strand breaks (53). However, AP sites can also be repaired by apurinic/apyrimidinic endonuclease 1 (APE1) during BER (38). APE1 initiates AP site repair by cleaving the phosphodiester backbone 5' to the AP site (41, 42, 53). APE1 creates a 3'-OH group and a 5'-deoxyribose phosphate residue so that a DNA polymerase can insert new nucleotides, and a DNA ligase can then join the strand (60). If APE1 fails to remove AP sites, the DNA damage response is initiated, with activation of histone H2A (phospho-H2AX) (38, 61). However, if cells are damaged beyond repair, p53 and its effector, p53-upregulated modulator of apoptosis (PUMA), elicit irreversible DNA fragmentation and apoptosis, so that dysfunctional cells are cleared from the organism (15).

Several studies have scrutinized APE1 in the context of cerebral ischemia. For example, APE expression has long been known to drop after ischemic injury (14, 24, 29, 34). The administration of an APE-mimicking peptide after reperfusion increases DNA repair, attenuates caspase-3 activity, and reduces infarct volume (32). Similarly, adenovirus-mediated APE1 upregulation reduces AP sites, 8-OHdG formation, DNA fragmentation, and infarct volume after ischemia/reperfusion injury (31). A previous study of ours demonstrated that pituitary adenylate cyclase activating polypeptide (PACAP) protects neurons against ischemia in an APE1-dependent manner (57). We discovered that APE1 was raised by PACAP through protein kinase A- and p38-dependent phosphorylation of cyclic adenosine monophosphate response element binding protein and activating transcription factor 2. Knockdown of APE1 attenuated the PACAP-induced protection against ischemia. However, none of these previous studies have specifically examined whether APE1 preserves long-term neurological function after ischemia. Moreover, the effect of APE1 on synaptic structures and their electrophysiological function, two features that define neuronal viability, is also unknown. Therefore, the goal of the present study was to thoroughly characterize the protective phenotype of APE1 upregulation *in vivo*. APE1 expression was increased in the rat hippocampus by three means: (1) preconditioning with repetitive low-dose proton irradiation (PI); (2) classical preconditioning with short-duration ischemic episodes; and (3)

transgenic APE1 overexpression. Using the first two models, we tested the hypothesis that APE1 mediates the protective effects of mild stress-induced preconditioning.

In addition to its role as a DNA repair protein, APE1 also acts as a reduction-oxidation factor and is known as redox factor-1. In this role, APE1 maintains many transcription factors in an active, reduced state. APE1 can stimulate the DNA binding ability of activator protein-1 proteins, nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), and hypoxia-inducible factor-1 α , and modulate the activity of p53 (23). APE1 also controls reactive oxygen species by negatively regulating the activity of the GTPase Rac1 (58). Thus, APE1 governs a number of cellular processes other than DNA repair. To determine whether APE1-induced neuroprotection against cerebral ischemia is mediated by its DNA repair capacity, we compared the impact of a repair-incompetent/redox-competent mutation (D210A) in APE1 with fully functional, wild-type APE1. Using these functionally distinct constructs, the present study reveals a central role of DNA repair in APE1-induced neuroprotection after cerebral ischemia.

Results

PI preconditioning raises APE1 and protects hippocampal CA1 neurons against ischemia

Ionizing radiation is known to raise APE1 mRNA and protein levels in a dose-dependent fashion (19). Therefore, we hypothesized that low-dose PI of the brain would upregulate APE1 and precondition CA1 neurons in the dorsal hippocampus against transient global cerebral ischemia (tGCI). The optimal parameters for PI-induced BER engagement and neuroprotection are shown in Supplementary Figure S1 (Supplementary Data are available online at www.liebertpub.com/ars). Rats were subjected to 1–5 days of PI (2 Gray [Gy]/day). One day after the last irradiation, sham controls or irradiated animals were subjected to 12 min of tGCI and sacrificed 3 days later. Four repetitions of PI (2 Gy/day) elicited maximal neuroprotection against CA1 neuronal death (Supplementary Fig. S1A). As expected, PI for 4 days increased BER-related proteins, such as 8-oxoguanine glycosylase (OGG1), APE1, and, to a lesser extent, β -polymerase (β -pol, Supplementary Fig. S1B–D). DNA ligase I and III, and X-ray repair cross-complementing protein 1 (XRCC1) were not affected. Immunofluorescence analysis revealed that 4 days of PI induced a higher expression of APE1 within CA1 neurons that were dual labeled with the neuronal phenotypic marker NeuN (Supplementary Fig. S1C). *In situ* autoradiography also revealed that APE1 mRNA was raised by 4 days of PI in multiple brain regions, including the hippocampus and cortex (Supplementary Fig. S1D). However, APE1 was not robustly increased by 1 day of PI. These findings suggest that the protection elicited by PI correlates with sizeable inductions of APE1.

Based on these findings, rats were subjected to an optimal dose of 2 Gy/day for 4 days in subsequent experiments. In non-irradiated animals, there was extensive destruction of CA1 neurons in response to 8–12 min of tGCI (Fig. 1A). As expected, PI protected against CA1 cell loss in all groups. PI also decreased tGCI-induced DNA fragmentation and oxidative damage to DNA bases, as shown by terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) staining (Fig. 1B) and 8-OHdG formation

FIG. 1. PI preconditioning protects against ischemia-induced cell loss in the hippocampal CA1 sector and reduces DNA damage. (A) Rats were subjected to PI preconditioning (2 Gy/day for 4 days) or sham irradiation. Twenty-four hours after the last irradiation, animals were subjected to 8, 10, or 12 min of tGCI or sham surgery. Viable CA1 neurons in the dorsal hippocampus were counted by stereology in hematoxylin and eosin-stained sections 3 days after tGCI or after sham surgery. (B) TUNEL staining for fragmented DNA in CA1 was performed on the same groups of animals as in (A). (C, D) Oxidative DNA damage markers 8-OHdG and AP sites were measured in DNA extracts from the hippocampal CA1 sector at 8 or 24 h after 12 min tGCI or sham surgery (S). $n=8$ animals/group. * $p \leq 0.05$, ** $p \leq 0.01$ PI preconditioned *versus* sham-irradiated rats, Bonferroni/Dunn's *post hoc* after ANOVA. 8-OHdG 8-hydroxy-2'-deoxyguanosine; ANOVA, analysis of variance; AP, apurinic/aprimidinic; Gy, gray; PI, proton irradiation; tGCI, transient global cerebral ischemia; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling.

(Fig. 1C), respectively. In addition, PI reduced AP site accumulation (Fig. 1D). These results reveal that PI increases DNA repair capacity and protects hippocampal neurons against ischemia *in vivo*.

APE1 induction mediates the neuroprotective effect of PI and ischemic preconditioning

So far, we showed a strong correlation between APE1 induction and neuroprotection. We then used RNA interference to test the hypothesis that APE1 is essential for PI-induced neuroprotection. Lentiviral infection with a short hairpin RNA (shRNA) sequence targeting APE1 significantly attenuated APE1 induction by PI preconditioning (Fig. 2A). In proton-irradiated animals infused with the control lentivirus, APE1 immunostaining overlapped with green fluorescent protein (GFP) expression within CA1 neurons (Fig. 2B[b, c]). However, in animals infused with the targeting sequence, APE1 expression within GFP-positive neurons was lowered (Fig. 2B[e, f]). As expected, APE1 knockdown prevented the protective impact of PI on CA1 neurons after tGCI (Fig. 2C). The same pattern was evident with a second shRNA sequence against a different portion of APE1 mRNA (data not shown). These studies confirm that endogenous APE1 upregulation mediates the protective impact of sub-lethal PI *in vivo*.

The neuroprotection elicited by PI is an example of endogenous defenses against mild cellular stress. Mild cellular

stress is known to engage the vitagene network of pro-survival proteins such as heat shock proteins 70 (Hsp70), Hsp32 (also known as heme oxygenase 1), thioredoxin, and sirtuin-1 (8, 10, 20). In order to examine whether PI also increased vitagenes, we probed for Hsp32, Hsp70, thioredoxin, and sirtuin-1 (Supplementary Fig. S2). Although thioredoxin and sirtuin-1 were not increased by PI, both Hsps were upregulated. These findings suggest that PI engages part of the vitagene network.

Another example of endogenous defenses is the neuroprotection elicited by classical ischemic preconditioning. Previous studies have shown that sub-lethal ischemic preconditioning upregulates the BER pathway (37). Therefore, we tested the hypothesis that APE1 may also mediate the protective effects of ischemic preconditioning. Animals were infused with lentiviral particles targeting APE1 or bearing the scrambled sequence. Fourteen days later, ischemic preconditioning was elicited by two short-duration (2 min) tGCI treatments on consecutive days. This was followed by a longer-duration (12 min) tGCI 2 days later. First, we verified that APE1 was upregulated by ischemic preconditioning and that it was knocked down by the targeting sequence (Supplementary Fig. S3A). Ischemic preconditioning robustly protected CA1 neurons against ischemic injury 3 days after tGCI, and APE1 knockdown partially reversed this protective effect (Supplementary Fig. S3B). Along with the PI studies, these data reveal that mild stress upregulates APE1 and members of the vitagene

FIG. 2. APE1 knockdown prevents preconditioning-afforded neuroprotection against ischemia in hippocampal CA1. (A) Animals were infused with empty lentiviral particles (Lenti), lentiviral particles with a control, scrambled sequence (APE1sc), or lentiviral particles targeting APE1 (APE1t). Fourteen days later, rats were proton-irradiated with 2 Gy/day for 4 days and then sacrificed 1 day after preconditioning. Western blots for APE1 and β -actin were performed on hippocampal tissue. (B) Immunofluorescence revealed loss of APE1 (red) within GFP-containing CA1 neurons (green) after lentiviral-GFP APE1t infusions. Arrows in (a) and (d) point to CA1 sector, and (b) and (e) show higher-power views of CA1. Arrows in (c) and (f) point to GFP⁺ neurons that are dual labeled with APE1 (yellow signal) in (c) but contain less APE1 in (f). (C) Animals were subjected to PI preconditioning as described earlier, followed by 12 min tGCI or sham surgery on the next day and sacrificed 3 days later. Viable CA1 neurons in the dorsal hippocampus were counted by stereology. $n = 8$ animals/group. $*p \leq 0.05$, $**p \leq 0.01$ for bracketed comparisons, Bonferroni/Dunn's *post hoc* after ANOVA. APE1, apurinic/aprimidinic endonuclease 1; APE1sc, scrambled shRNA sequence of APE1; APE1t, shRNA sequence targeting APE1; GFP, green fluorescent protein. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

network, and that APE1 partially mediates its neuroprotective effects.

OGG1 does not contribute to PI-mediated neuroprotection

OGG1 is a DNA glycosylase enzyme that is involved in BER. OGG1 and its target, 8-OHdG, are biomarkers for oxidative DNA damage in many pathological conditions, including ischemia (40, 66). As mentioned earlier, we found that PI preconditioning increased OGG1 in the CA1 sector and reduced the accumulation of 8-OHdG. Thus, we tested the hypothesis that OGG1 contributes to PI preconditioning against tGCI (Fig. 3). In contrast to APE1 knockdown, OGG1 knockdown in CA1 did not prevent PI-induced neuroprotection 3 days after tGCI (Fig. 3B). These findings suggest that OGG1 and oxidized bases do not play critical roles in ischemic cell death, at least not within 3 days after tGCI.

APE1 overexpression protects hippocampal CA1 neurons against ischemic injury

Next, we tested the hypothesis that APE1 overexpression in transgenic rats (Tg-APE1) would protect against tGCI. Electroencephalographic (EEG) recordings were performed on all animals and verified that Tg-APE1 rats exhibited the same electrocerebral silence during tGCI as did wild-type controls (Fig. 4A). This control experiment confirms that both groups of rats were exposed to the same initial insult. Extensive loss of hematoxylin and eosin-stained CA1 neurons was observed in wild-type animals (see black arrows in Fig. 4B[a, c]) after tGCI. In contrast, Tg-APE1 rats exhibited less dramatic neuronal loss at both 3 and 28 days after tGCI (Fig. 4B[b, d]). Stereological counts of viable CA1 neurons confirmed that there was no change in the basal number of neurons under sham conditions in transgenic animals (Fig. 4C). As expected, Tg-APE1 rats were significantly protected against CA1 cell loss at 72 h and 28 days after tGCI and exhibited fewer DNA-fragmented neurons

FIG. 3. Knockdown of OGG1 does not prevent preconditioning-induced neuroprotection of CA1 after ischemia. (A) Western immunoblots of OGG1 in the hippocampus of naïve control rats or proton-irradiated rats (2 Gy/day for 4 days) that were infused with empty lentiviral vectors (Lenti), scrambled control lentiviral shRNA vectors (OGG1sc), or lentivirus shRNA particles targeting OGG1 (OGG1t). Animals were sacrificed 1 day after PI. **(B)** The same groups as in **(A)** were treated with 12 min tGCI on the day after the final PI and sacrificed 3 days later for stereological cell counts of viable CA1 neurons. $**p \leq 0.01$ for bracketed comparisons, Bonferroni/Dunn's *post hoc* after ANOVA. OGG1, 8-oxoguanine glycosylase. n.s., not significant.

(Fig. 4D). Similarly, Tg-APE1 rats exhibited less accumulation of AP sites over time after tGCI (Fig. 5A). These findings reveal that APE1 overexpression protects hippocampal neurons against ischemic injury and DNA damage *in vivo*.

APE1 overexpression reduces the DNA damage response

DNA damage is known to elicit a robust DNA damage response, as measured by activation of H2AX (γ -H2AX, phosphorylated at Ser139) and induction of PUMA (28, 45). The DNA damage response to tGCI was attenuated by APE1 overexpression, as illustrated by decreased levels of phospho-H2AX and PUMA at 24 h post-injury (Fig. 5B, C). Phospho-H2AX is usually diffuse and present at low levels, but it was transformed into a punctate, nuclear pattern in CA1 neurons after tGCI (Fig. 5D). Although there was no impact of APE1 on H2AX phosphorylation 8 h after ischemia, APE1 transgenics exhibited less phospho-H2AX staining by 24 h, closely following the immunoblot data. The DNA repair process, therefore, appeared to be more efficient in transgenics. Cytosolic PUMA levels rose after tGCI, presumably clustering along mitochondria, but this effect was also prevented in APE1 overexpressors (Fig. 5E). These results suggest that APE1 overexpression attenuates the robustly induced DNA damage response after ischemia.

APE1 overexpression preserves synaptic markers and electrophysiological function

The impact of APE1 on synaptic structures and their electrophysiological function is not known. Therefore, we tested the hypothesis that APE1 would protect these critical measures of neuronal viability against ischemic injury. As expected, tGCI elicited severe deformation and loss of Golgi-impregnated neuronal structures and their neuritic processes in wild-type rats at 1 day and 3 days post-injury

(Fig. 6A–C). In contrast, APE1 overexpression prevented dendritic beading and the loss of mature apical dendritic spines (Fig. 6B, C). APE1 also restored spine length at 1 day after tGCI. Three days after ischemia, the loss of spines and spine length was still less dramatic in Tg-APE1 rats than in wild-type controls, indicating partial preservation of synaptic structures. Next, we measured synaptophysin-1, a marker for presynaptic vesicles, and postsynaptic density protein-95 (PSD-95), a postsynaptic protein. Extensive loss of synaptophysin-1 and PSD-95 was evident in wild-type rats at 3 and 28 days after tGCI (Fig. 7A–D). In contrast, synaptophysin-1 and PSD-95 levels were partially preserved in transgenic rats for approximately 28 days post-injury. Immunofluorescence analyses confirmed these immunoblot findings (Supplementary Fig. S4). In order to extend these findings to the functional level, we examined the electrophysiological properties of hippocampal synapses by stimulating the Schaffer collateral pathway and recording from CA1. Field excitatory postsynaptic potentials (fEPSPs) in CA1 were greatly reduced in wild-type rats at 3 and 28 days after ischemia, indicating a loss of synaptic function (Fig. 7C, D). In comparison, Tg-APE1 rats exhibited less severe loss of fEPSPs, which was consistent with the Golgi stain and with the partial preservation of synaptophysin and PSD-95. These findings demonstrate that APE1 helps preserve both structural and functional properties of CA1 neurons soon after ischemia and that this partial preservation is long lasting.

APE1 overexpression prevents neurological deficits after ischemia

We examined spatial learning and memory deficits in the Morris water maze in order to test the hypothesis that APE1 elicits long-term preservation of neurological function. At 22–26 days after sham surgery, there was no difference between wild-type animals and APE1 transgenics in the ability

FIG. 4. Transgenic APE1 overexpression protects against ischemia-induced cell loss in hippocampal CA1 and blunts DNA fragmentation. (A) Electrical activity was comparably flattened by tGCI in WT rats and transgenic APE1 overexpressors, as shown by representative EEG recordings. (B) Hematoxylin and eosin-stained sections through the dorsal hippocampus at 3 and 28 days after 12 min tGCI or sham surgery. Black arrows denote CA1 sector (a–d). (C) Stereological counts of viable CA1 neurons in WT or Tg-APE1 rats subjected to sham surgery 3 days earlier. (D) Stereological counts of viable CA1 neurons in WT or Tg-APE1 rats subjected to 12 min tGCI 3 or 28 days earlier. TUNEL-labeled CA1 neurons in the same rats are shown on the right. $n=8-10$ animals/group. $**p \leq 0.01$, $***p \leq 0.001$ WT versus Tg-APE1 rats, Bonferroni/Dunn's *post hoc* after ANOVA. EEG, electroencephalography; Tg-APE1, transgenic rats overexpressing APE1; WT, wild-type. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

to locate the submerged platform (learning phase) or to remember its location once it was removed (memory phase) (Fig. 8A). However, after ischemia, wild-type rats spent more time than APE1 transgenics in all four pool quadrants during both the learning and memory phases of the test (Fig. 8B). Swim speed was unaffected by transgene expression after sham surgery or tGCI, illustrating that wild-type and transgenic rats had equivalent motor skills (Fig. 8C). The latency to find the submerged platform after sham surgery was also similar for wild-type animals and APE1 transgenics, illustrating no change in baseline learning and memory skills (Fig. 8D). However, the latency to find the submerged platform was significantly reduced in transgenics 24–26 days after tGCI (Fig. 8E). Spatial memory of the submerged platform once it was removed from the pool was also significantly improved in transgenic rats at 27 days after tGCI, although there was no impact on this measure in sham controls (Fig. 8F). These results demonstrate long-term preservation of neurological function with APE1 up-regulation.

Loss of the DNA repair function of APE1 abolishes neuroprotection against ischemia

Finally, we tested the hypothesis that the DNA repair function of APE1 is essential for neuroprotection. First, we contrasted transgenic overexpression of wild-type APE1 (Tg-APE) with overexpression of the repair-incompetent/redox-competent mutation D210A (Tg-APE-D210A) or a repair-competent/redox-incompetent C65A mutation (Tg-APE-C65A; Supplementary Fig. S5). Previous studies have shown that D210A reduces the DNA repair activity of APE by 25,000-fold (22) and that C65A disrupts the redox active site (63). Tg-APE rats and rats with mutant APE1 genes expressed elevated levels of APE mRNA and protein, as shown by *in situ* autoradiography, immunohistochemistry, and Western blotting (Supplementary Fig. S5C, D). An oligonucleotide incision assay demonstrated specific APE activity in the hippocampus of Tg-APE and Tg-APE-C65A rats (Supplementary Fig. S5E). In contrast, DNA repair activity was low in Tg-APE-D210A rats. Tg-APE and Tg-APE-C65A

FIG. 5. Transgenic APE1 overexpression attenuates the DNA damage response in hippocampal CA1 after ischemia. (A) AP site frequency was measured in WT and Tg-APE1 rats under sham conditions (S) or 8, 24, and 48 h after 12 min tGCI. (B) Phospho-H2AX (p-H2AX), total H2AX, and PUMA levels were assessed in hippocampal CA1 protein extracts by Western blotting 8 and 24 h after 12 min tGCI. Immunoblot results are quantified in (C). (D) Double-label immunofluorescent staining for p-H2AX and propidium iodide (PI) in CA1 in the same groups as shown in (B) and (C). (E) Immunofluorescent staining for PUMA (green) and NeuN-labeled neuronal nuclei (red) in CA1 in similar groups as shown in (B) and (C). Nuclei were labeled blue with Hoechst. $n=6$ animals/group. $*p \leq 0.05$, $**p \leq 0.01$, WT versus Tg-APE1 rats, Bonferroni/Dunn's *post hoc* after ANOVA. H2AX, histone H2A; PUMA, p53 upregulated modulator of apoptosis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

rats also exhibited increases in total BER activity (Supplementary Fig. S5F). In contrast, Tg-APE-D210A rats exhibited minimal BER activity. Next, we tested the hypothesis that the D210A mutation would eliminate the ability of APE1 to reduce AP sites after ischemia. We measured AP sites after tGCI and found a greater increase in Tg-APE-D210A rats at 8 h (Fig. 9A). These results suggest that overexpression of APE-D210A interfered with the DNA repair activity of endogenous APE1, presumably by competing for binding to DNA substrates. As expected, no protective impact of the D210A mutation on viable CA1 neurons or DNA fragmentation after tGCI was observed (Fig. 9B, C). Thus, loss of DNA repair activity not only exacerbates AP site formation, but also abrogates the ability of APE1 to protect against ischemia.

Discussion

The present study characterizes the protective impact of APE1 upregulation in a rat model of tGCI. In contrast to previous studies, we examined multiple phenotypes of APE1 upregulation, ranging from synaptic structures and electrophysiological functions to neurological integrity. Our study is also novel in that we raised APE1 by three means: natural induction of APE1 by sub-lethal DNA damage from PI and by classical ischemic preconditioning, as well as artificial overexpression of APE1 mRNA and protein from a transgene. Both endogenous and transgenic upregulation of APE1 partially rescued neuronal structures in the CA1 sector of the rat hippocampus. AP site accumulation, oxidative DNA base damage, and DNA fragmentation were also ameliorated.

FIG. 6. Transgenic APE1 overexpression protects hippocampal CA1 dendrites from ischemia-induced retraction. **(A)** Representative images of Golgi-stained sections of the dorsal hippocampus and cortex in WT rats under sham conditions and in WT and Tg-APE1 rats 72h after 12 min tGCI. Images were photographed at 4×(cortex plus hippocampus) or 10×(hippocampus) magnification. **(B)** Representative images of apical dendrites in Golgi-stained CA1 pyramidal neurons, 24 or 72h after tGCI, or after sham surgery. Arrows point to dendritic beadings, a hallmark of injured dendrites. Stars point to mature dendritic spines. **(C)** Quantification of number of spines per unit length and spine length in the same animals as shown in **(A)** and **(B)**. $n=3-5$ animals/group. $*p \leq 0.05$, $**p \leq 0.01$ for bracketed comparisons, Bonferroni/Dunn's *post hoc* after ANOVA.

FIG. 7. Transgenic APE1 overexpression protects hippocampal CA1 neurons against ischemia-induced loss of synaptic proteins and electrophysiological function. **(A)** Western blots for SYP1 and PSD-95 after sham surgery (S) or 3 and 28 days after 12 min tGCI in WT animals or Tg-APE1 overexpressors. Protein levels are quantified in **(B)**. fEPSP I/O slope in CA1 neurons 3 days **(C)** or 28 days **(D)** after 12 min tGCI or after sham surgery. The intensity of stimulation was systematically increased, and I/O slope was plotted as a percentage of the response at 300 μA. $n=8$ animals/group. $*p \leq 0.05$, $**p \leq 0.01$, WT versus Tg-APE1 rats, Bonferroni/Dunn's *post hoc* after ANOVA. fEPSP, field excitatory postsynaptic potential; I/O, input/output; PSD-95, postsynaptic density protein-95; SYP1, synaptophysin 1.

FIG. 8. Transgenic APE1 overexpression protects long-term spatial learning and memory after ischemia. (A) The Morris water maze test was performed on WT and Tg-APE1 rats 22–27 days after sham surgery. Shown are representative images of the swim paths of rats in each group while the submerged platform was still present (learning phase) and after it was removed (memory phase). (B) Representative images of the swim paths of wild-type and Tg-APE1 rats after 12 min tGCI. (C) Swim speed was measured in wild-type and Tg-APE1 rats subjected to sham surgery or tGCI 26 days earlier. (D) Time to locate the submerged platform was measured in WT and Tg-APE1 rats 22–26 days after sham surgery. (E) Time to locate the submerged platform was measured in WT and Tg-APE1 rats 22–26 days after tGCI. (F) Spatial memory of the location of the previously submerged platform was measured in WT and Tg-APE1 rats 27 days after tGCI or sham surgery. Spatial memory is expressed as the percentage of time spent in the goal quadrant when the platform was removed. $n=8$ animals/group. $*p \leq 0.05$, $**p \leq 0.01$, WT versus Tg-APE1 rats, Bonferroni/Dunn's *post hoc* after ANOVA. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

APE1 blunted the increase in H2AX activation and in PUMA induction by inhibiting the DNA damage response. The numbers of dendritic spines and spine lengths in CA1 were partially preserved in transgenic overexpressors, as were pre- and post-synaptic proteins. Furthermore, the electrophysiological function of CA1 was less severely affected by ischemia in APE1 transgenics than in wild-type rats. Notably, APE1 up-regulation helped improve spatial learning and memory, as a positive impact of APE transgene expression was evident for 27 days post-injury in the Morris water maze. Finally, we found that the DNA repair function of APE1 was essential

for neuroprotection. On the basis of these findings, we can conclude that APE1 is a rational therapeutic target that helps improve neurological recovery from ischemic brain injury.

As mentioned in the "Introduction," DNA damage can be active or passive. Active DNA damage is mediated by endonucleases such as caspase-activated deoxynuclease, apoptosis-inducing factor, and endonuclease G (38). Passive DNA damage is endonuclease independent and involves both direct and indirect attacks by reactive oxygen species. Indirect damage to DNA can arise from oxidative damage to

FIG. 9. The DNA repair capacity of APE1 is essential for neuroprotection against ischemia. AP sites (A), viable CA1 neurons (B), and TUNEL-positive neurons (C) were measured after sham surgery (S) or after 12 min tGCI in WT animals and in transgenics with a DNA repair-incompetent/redox-competent mutation in the APE1 gene (Tg-D210A). AP sites were measured 8 and 24 h post-injury, whereas CA1 neuron counts and TUNEL staining were performed 3 days post-injury. $n=8$ animals/group. $*p \leq 0.05$, WT versus Tg-D210A rats, Bonferroni/Dunn's *post hoc* after ANOVA.

neighboring lipids and proteins (44, 65). In contrast, hydroxyl radicals can directly add protons to or extract hydrogen atoms from DNA. Oxidative damage to DNA elicits programmed cell death if the destruction is beyond repair. For example, we have found that AP sites are especially prevalent in those ischemic neurons that go on to develop fragmented DNA and die (17, 34). Irreparable oxidative DNA damage triggers death through poly(ADP-ribose) polymerase-1 activation, nicotinamide adenine dinucleotide depletion, apoptosis-inducing factor release, c-Jun NH₂-terminal kinase, p53, and PUMA (36, 38). PUMA and H2AX were assessed in this study, because they are highly sensitive to DNA damage. Activation of H2AX by ataxia telangiectasia mutated protein is an early cellular response to strand breaks in DNA (45) and hastens cellular recovery by increasing DNA accessibility and facilitating the recruitment of other DNA damage response players (45, 54). In contrast to the pro-survival role of H2AX, PUMA forms an integral part of p53-mediated cell death in ischemic injury and elicits mitochondrial outer membrane permeabilization and caspase-mediated apoptosis (27, 28). In the present study, APE1 prevented both H2AX activation and PUMA induction, suggesting that it preserved DNA integrity. This conclusion is also supported by our direct measurements of AP sites, DNA fragmentation, and 8-OHdG formation.

In contrast to the apoptotic response to lethal DNA damage, BER likely evolved as an essential compensatory response to the high frequency of sub-lethal damage in DNA (38). Even under physiological conditions, AP sites arise at a rate of 50,000–200,000 per cell per day (50). Many proteins other than APE1 also participate in BER. For example, OGG1 recognizes and catalyzes the removal of 8-OHdG (30, 67), and XRCC1 forms repair complexes with β -pol and stimulates DNA ligase III, acting as a scaffold protein (13). Preconditioning with sub-lethal ischemia increases XRCC1, β -pol, and DNA ligase III and enhances BER activity (37). In the present study, both OGG1 and β -pol were increased by PI preconditioning, in addition to the robust induction of APE1. However, we did not gather evidence that oxidized DNA bases directly trigger ischemic cell death, at least not within the short time frame examined, because OGG1 knockdown did not impact the neuroprotection afforded by PI preconditioning. Future studies examining the long-term impact of OGG1 knockdown in ischemia are therefore warranted. In contrast, the APE1 knockdown and overexpression data suggest that ischemia-induced AP sites are lethal to hippocampal neurons within short time frames.

Although it has been known for many years that active DNA repair promotes cell survival (26, 34, 39), most studies of the association of APE1 with neuroprotection have been correlational in nature (25, 34, 39, 51). For example, studies have shown that APE1 expression is lowered by ischemia before cell death (14, 21, 24, 29, 34). APE1 levels decrease after both transient cerebral ischemia (24, 29) and traumatic brain injury (35), generally preceding DNA fragmentation. Furthermore, neuronal adenosine triphosphate loss during injury is accompanied by nuclear APE1 depletion (56). Conversely, higher APE1 expression is correlated with ischemic preconditioning and protective CuZn superoxide dismutase overexpression (39, 51). A few studies, mostly outside the field of stroke, have mechanistically linked APE1 to protection against cell death. For example, APE1 gene knockout is lethal to the embryo due to severe deficiencies in neurogenesis (43).

APE1 has been shown to be neuroprotective *in vitro* after ionizing radiation (62) and oxidative stress (61). Thus, APE1 deficiency exacerbates the negative impact of both oxidative injury and DNA damaging agents (48, 52, 61). Two studies by Kim *et al.* have shown that APE1 upregulation decreases ischemic injury *in vivo* (31, 32). Consistent with the present findings, these authors showed decreases in infarct volume, AP sites, 8-OHdG, and DNA fragmentation. The present study extends these previous findings to demonstrate that APE1 upregulation prevents ischemic injury, specifically through its DNA repair function, and that it preserves the structure and function of hippocampal neurons down to the synaptic level. Studies on the C65A mutation are currently underway to determine whether the redox activity of APE1 is less instrumental in neuroprotection than its DNA repair activity.

The present study is the first that demonstrates that PI and sub-lethal ischemic preconditioning protect CA1 neurons against ischemic injury in an APE1-dependent manner. A previous study on the myocardium revealed that ischemic preconditioning elicits nuclear translocation of thioredoxin-1, where it associates with APE1 and activates NF κ B and Akt signaling (46). Ischemic preconditioning was also shown to increase APE1 in the brain, although it was not known whether this was causally linked to neuroprotection (1, 39). Mild stressors such as sub-lethal ischemic episodes and PI are also known as hormetic stimuli. Hormesis is defined as favorable reactions to low-level stress and has been observed in the toxicological literature for many decades (4–7, 47). Hormesis elicits U-shaped or biphasic responses to stress and is mediated, at least in part, by the co-ordinated network of vitagenes (8–10, 20). Preconditioning increases the expression of the vitagenes thioredoxin, sirtuin-1, Hsp32, and Hsp70 (1, 2, 16, 59). In the present study, we also found that Hsp32 and Hsp70 were increased by PI preconditioning. Vitagenes regulate cellular fitness and lifespan by integrating information flow along convergent and divergent pathways into a cohesive stress response. Vitagenes may prevent diseases and promote longevity by sensing injurious stimuli, such as reactive oxygen species, and attenuating their impact. Our findings suggest that APE1 may also be included in this network of genes, as it is raised by low-dose stress and confers robust neuroprotection. In other words, APE1 is not likely to act in isolation, but probably exerts neuroprotection as a part of a co-ordinated group of stress-responsive molecules.

In conclusion, the present findings illustrate the utility of targeting BER in ischemia. Our findings are also consistent with case-control human studies on single nucleotide polymorphisms in the APE1 gene, as the G–C–T haplotype was found to be a genetic marker for cerebral infarction (49). Therefore, further mechanistic research into the BER pathway in humans is justified.

Materials and Methods

Methodological details beyond the descriptions given next are provided in the “Supplementary Methods” section. Stroke Treatment Academic Industry Roundtable guidelines were followed in all experiments. Animals were randomly assigned into experimental groups. EEG recordings were performed on all animals that underwent tGCI to ensure isoelectricity across groups. Blood pH, gases, and glucose during and after tGCI

were not affected by transgene expression (data not shown). Both rectal and temporalis muscle temperature were maintained between 36.5°C and 37.0°C during and after tGCI in all animals. Animals that did not exhibit electrocerebral silence or that died were excluded from further experimental procedures or data analyses (<10% of total numbers). All measurements and analyses were blinded.

Experimental ischemia and PI

Animal use was in accordance with the NIH Guide and approved by the University of Pittsburgh and Fudan University Institutional Animal Care and Use Committees. tGCI was induced for 12 min (unless indicated otherwise) under 1.5% isoflurane anesthesia in male Sprague–Dawley rats with the four-vessel occlusion method (18). For PI, the brain was focally irradiated with 2 Gy/day for 1–5 days under 1.5% isoflurane anesthesia. One day after the last irradiation, the rats were subjected to tGCI as described earlier. Ischemic preconditioning was performed with two short-duration tGCI episodes (2 min each) on two consecutive days, followed by 12 min of tGCI 48 h after the last preconditioning episode. Three and 28 days after tGCI, viable CA1 neurons in the hippocampus were counted by stereology as previously described (12).

shRNA vectors and transgenic animals

shRNA sequences against rat APE1 and OGG1 were inserted into lentiviral transfer vectors FSW under control of the U6 promoter (12, 57). The scrambled sequence was used as the control. Lentiviral vectors were infused into CA1 of the dorsal hippocampus using a convection-enhanced microinfusion system (12, 57). Transgenic APE1 overexpressors were generated in collaboration with the Transgenic Service Core at the University of Michigan. The chimeric transgene that we used to create the transgenic rats contains human APE cDNA under the control of the cytomegalovirus enhancer and a chicken β -actin promoter. A hemagglutinin tag was added before the APE1 coding region. Three types of rats were created: (1) Tg-APE1 (overexpressing wild-type APE1); (2) Tg-APE-C65A (overexpressing mutant APE1 with a deletion in transcription-related redox activity by altering C65 to alanine); and (3) Tg-APE-D210A (overexpressing mutant APE1 with reduced DNA repair activity by altering D210 to alanine).

Morris water maze

The Morris water maze was performed 22–27 days after tGCI, as previously described (64). Briefly, an 11 cm-diameter platform was submerged in a pool of opaque water. Rats were placed into the pool from one of four locations and allowed 90 s to locate the platform (learning phase). Latency to find the submerged platform was recorded for every trial. Four trials per day were performed for five consecutive days, followed by a single 60 s probe trial in which the platform was removed and time spent in the goal quadrant (memory phase) and swim speed were recorded.

Electrophysiology

Electrophysiological recordings of CA1 were made with modifications to previously reported techniques (3). Briefly, fEPSPs were evoked with a bipolar tungsten-stimulating electrode by orthodromic stimulation of CA3 pyramidal cell

axons in the Schaffer collateral pathway. Glass micropipettes filled with artificial cerebrospinal fluid (resistance 1–3 mega Ω) were used to measure fEPSPs in the recipient CA1 neurons in the stratum radiatum. The stimulus intensity was varied from 12.5 to 300 μ A, and the CA1 fEPSP input/output slope was expressed as a percentage of the response at 300 μ A.

DNA damage assays

AP sites were measured with the biotin-labeled aldehyde reactive probe in a colorimetric assay (Dojindo Molecular Technologies), as described previously (57). TUNEL staining was performed with a kit according to the manufacturer's instructions (Roche Applied Science). 8-OHdG measurements of oxidative DNA damage were made as previously described (33).

Statistics

Experiments were performed on eight animals per group, unless stated otherwise. Data are reported as mean $\pm$ standard error of the mean. The significance of the difference between means was assessed by the two-tailed *t*-test for single comparisons and by the Bonferroni/Dunn's *post hoc* test following analysis of variance for multiple comparisons. $p \leq 0.05$ was deemed significantly different.

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Author Disclosure Statement

No competing financial interests exist.

References

- Andoh T, Chock PB, and Chiueh CC. Preconditioning-mediated neuroprotection: role of nitric oxide, cGMP, and new protein expression. *Ann N Y Acad Sci* 962: 1–7, 2002.
- Bergeron M, Ferriero DM, Vreman HJ, Stevenson DK, and Sharp FR. Hypoxia-ischemia, but not hypoxia alone, induces the expression of heme oxygenase-1 (HSP32) in newborn rat brain. *J Cereb Blood Flow Metab* 17: 647–658, 1997.
- Bervers MB, Ingleton LP, Che D, Cole JT, Li L, Da T, Kopil CM, Cohen AS, and Neumar RW. RNAi targeting micro-calpain increases neuron survival and preserves hippocampal function after global brain ischemia. *Exp Neurol* 224: 170–177, 2010.
- Calabrese EJ. Converging concepts: adaptive response, preconditioning, and the Yerkes-Dodson Law are manifestations of hormesis. *Ageing Res Rev* 7: 8–20, 2008.
- Calabrese EJ. Hormesis is central to toxicology, pharmacology and risk assessment. *Hum Exp Toxicol* 29: 249–261, 2010.
- Calabrese EJ, Bachmann KA, Bailer AJ, Bolger PM, Borak J, Cai L, Cedergreen N, Cherian MG, Chiueh CC, Clarkson TW, Cook RR, Diamond DM, Doolittle DJ, Dorato MA,

- Duke SO, Feinendegen L, Gardner DE, Hart RW, Hastings KL, Hayes AW, Hoffmann GR, Ives JA, Jaworowski Z, Johnson TE, Jonas WB, Kaminski NE, Keller JG, Klaunig JE, Knudsen TB, Kozumbo WJ, Lettieri T, Liu SZ, Maisseu A, Maynard KI, Masoro EJ, McClellan RO, Mehendale HM, Mothersill C, Newlin DB, Nigg HN, Oehme FW, Phalen RF, Philbert MA, Rattan SI, Riviere JE, Rodricks J, Sapolsky RM, Scott BR, Seymour C, Sinclair DA, Smith-Sonneborn J, Snow ET, Spear L, Stevenson DE, Thomas Y, Tubiana M, Williams GM, and Mattson MP. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicol Appl Pharmacol* 222: 122–128, 2007.
7. Calabrese EJ and Blain RB. The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. *Regul Toxicol Pharmacol* 61: 73–81, 2011.
 8. Calabrese V, Cornelius C, Cuzzocrea S, Iavicoli I, Rizzarelli E, and Calabrese EJ. Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. *Mol Aspects Med* 32: 279–304, 2011.
 9. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ, and Mattson MP. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxid Redox Signal* 13: 1763–1811, 2010.
 10. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Paola R, Koverech A, Cuzzocrea S, Rizzarelli E, and Calabrese EJ. Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. *Biochim Biophys Acta* 1822: 753–783, 2012.
 11. Canugovi C, Misiak M, Ferrarelli LK, Croteau DL, and Bohr VA. The role of DNA repair in brain related disease pathology. *DNA Repair (Amst)* 12: 578–587, 2013.
 12. Cao G, Xing J, Xiao X, Liou AK, Gao Y, Yin XM, Clark RS, Graham SH, and Chen J. Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury. *J Neurosci* 27: 9278–9293, 2007.
 13. Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, and Frosina G. Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *J Biol Chem* 272: 23970–23975, 1997.
 14. Chang YY, Fujimura M, Morita-Fujimura Y, Kim GW, Huang CY, Wu HS, Kawase M, Copin JC, and Chan PH. Neuroprotective effects of an antioxidant in cortical cerebral ischemia: prevention of early reduction of the apurinic/apyrimidinic endonuclease DNA repair enzyme. *Neurosci Lett* 277: 61–64, 1999.
 15. Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, Maier CM, Narasimhan P, Goeders CE, and Chan PH. Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. *Antioxid Redox Signal* 14: 1505–1517, 2011.
 16. Chen J, Graham SH, Zhu RL, and Simon RP. Stress proteins and tolerance to focal cerebral ischemia. *J Cereb Blood Flow Metab* 16: 566–577, 1996.
 17. Chen J, Jin K, Chen M, Pei W, Kawaguchi K, Greenberg DA, and Simon RP. Early detection of DNA strand breaks in the brain after transient focal ischemia: implications for the role of DNA damage in apoptosis and neuronal cell death. *J Neurochem* 69: 232–245, 1997.
 18. Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, and Simon RP. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. *J Neurosci* 18: 4914–4928, 1998.
 19. Chen S, Xiong G, Wu S, and Mo J. Downregulation of apurinic/apyrimidinic endonuclease 1/redox factor-1 enhances the sensitivity of human pancreatic cancer cells to radiotherapy *in vitro*. *Cancer Biother Radiopharm* 28: 169–176, 2013.
 20. Cornelius C, Perrotta R, Graziano A, Calabrese EJ, and Calabrese V. Stress responses, vitagenes and hormesis as critical determinants in aging and longevity: mitochondria as a “chi”. *Immun Ageing* 10: 15, 2013.
 21. Edwards M, Kent TA, Rea HC, Wei J, Quast M, Izumi T, Mitra S, and Perez-Polo JR. APE/Ref-1 responses to ischemia in rat brain. *Neuroreport* 9: 4015–4018, 1998.
 22. Erzberger JP and Wilson DM, 3rd. The role of Mg²⁺ and specific amino acid residues in the catalytic reaction of the major human abasic endonuclease: new insights from EDTA-resistant incision of acyclic abasic site analogs and site-directed mutagenesis. *J Mol Biol* 290: 447–457, 1999.
 23. Evans AR, Limp-Foster M, and Kelley MR. Going APE over ref-1. *Mutat Res* 461: 83–108, 2000.
 24. Fujimura M, Morita-Fujimura Y, Kawase M, and Chan PH. Early decrease of apurinic/apyrimidinic endonuclease expression after transient focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 19: 495–501, 1999.
 25. Fujimura M, Morita-Fujimura Y, Narasimhan P, Copin JC, Kawase M, and Chan PH. Copper-zinc superoxide dismutase prevents the early decrease of apurinic/apyrimidinic endonuclease and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. *Stroke* 30: 2408–2415, 1999.
 26. Guillet M and Boiteux S. Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in *Saccharomyces cerevisiae*. *EMBO* 21: 2833–2841, 2002.
 27. Hikisz P and Kilianska ZM. PUMA, a critical mediator of cell death—one decade on from its discovery. *Cell Mol Biol Lett* 17: 646–669, 2012.
 28. Hong LZ, Zhao XY, and Zhang HL. p53-mediated neuronal cell death in ischemic brain injury. *Neurosci Bull* 26: 232–240, 2010.
 29. Kawase M, Fujimura M, Morita-Fujimura Y, and Chan PH. Reduction of apurinic/apyrimidinic endonuclease expression after transient global cerebral ischemia in rats: implication of the failure of DNA repair in neuronal apoptosis. *Stroke* 30: 441–448; discussion 449, 1999.
 30. Kershaw RM and Hodges NJ. Repair of oxidative DNA damage is delayed in the Ser326Cys polymorphic variant of the base excision repair protein OGG1. *Mutagenesis* 27: 501–510, 2012.
 31. Kim HW, Cho KJ, Park SC, Kim HJ, and Kim GW. The adenoviral vector-mediated increase in apurinic/apyrimidinic endonuclease inhibits the induction of neuronal cell death after transient ischemic stroke in mice. *Brain Res* 1274: 1–10, 2009.
 32. Kim HW, Cho KJ, Lee BI, Kim HJ, and Kim GW. Post-ischemic administration of peptide with apurinic/apyrimidinic endonuclease activity inhibits induction of cell death after focal cerebral ischemia/reperfusion in mice. *Neurosci Lett* 460: 166–169, 2009.
 33. Lan J, Henshall DC, Simon RP, and Chen J. Formation of the base modification 8-hydroxyl-2'-deoxyguanosine and DNA fragmentation following seizures induced by systemic kainic acid in the rat. *J Neurochem* 74: 302–309, 2000.
 34. Lan J, Li W, Zhang F, Sun FY, Nagayama T, O'Horo C, and Chen J. Inducible repair of oxidative DNA lesions in the rat

- brain after transient focal ischemia and reperfusion. *J Cereb Blood Flow Metab* 23: 1324–1339, 2003.
35. Lewen A, Sugawara T, Gasche Y, Fujimura M, and Chan PH. Oxidative cellular damage and the reduction of APE1/Ref-1 expression after experimental traumatic brain injury. *Neurobiol Dis* 8: 380–390, 2001.
 36. Li H, Liu N, Rajendran GK, Gernon TJ, Rockhill JK, Schwartz JL, and Gu Y. A role for endogenous and radiation-induced DNA double-strand breaks in p53-dependent apoptosis during cortical neurogenesis. *Radiat Res* 169: 513–522, 2008.
 37. Li N, Wu H, Yang S, and Chen D. Ischemic preconditioning induces XRCC1, DNA polymerase-beta, and DNA ligase III and correlates with enhanced base excision repair. *DNA Repair (Amst)* 6: 1297–1306, 2007.
 38. Li P, Hu X, Gan Y, Gao Y, Liang W, and Chen J. Mechanistic insight into DNA damage and repair in ischemic stroke: exploiting the base excision repair pathway as a model of neuroprotection. *Antioxid Redox Signal* 14: 1905–1918, 2011.
 39. Li W, Luo Y, Zhang F, Signore AP, Gobbel GT, Simon RP, and Chen J. Ischemic preconditioning in the rat brain enhances the repair of endogenous oxidative DNA damage by activating the base-excision repair pathway. *J Cereb Blood Flow Metab* 26: 181–198, 2006.
 40. Lin LH, Cao S, Yu L, Cui J, Hamilton WJ, and Liu PK. Up-regulation of base excision repair activity for 8-hydroxy-2'-deoxyguanosine in the mouse brain after forebrain ischemia-reperfusion. *J Neurochem* 74: 1098–1105, 2000.
 41. Ljungquist S, Andersson A, and Lindahl T. A mammalian endonuclease specific for apurinic sites in double-stranded deoxyribonucleic acid. II. Further studies on the substrate specificity. *J Biol Chem* 249: 1536–1540, 1974.
 42. Ljungquist S and Lindahl T. A mammalian endonuclease specific for apurinic sites in double-stranded deoxyribonucleic acid. I. Purification and general properties. *J Biol Chem* 249: 1530–1535, 1974.
 43. Ludwig DL, MacInnes MA, Takiguchi Y, Purtymun PE, Henrie M, Flannery M, Meneses J, Pedersen RA, and Chen DJ. A murine AP-endonuclease gene-targeted deficiency with post-implantation embryonic progression and ionizing radiation sensitivity. *Mutat Res* 409: 17–29, 1998.
 44. Luxford C, Dean RT, and Davies MJ. Induction of DNA damage by oxidised amino acids and proteins. *Biogerontology* 3: 95–102, 2002.
 45. Mah LJ, El-Osta A, and Karagiannis TC. GammaH2AX as a molecular marker of aging and disease. *Epigenetics* 5: 129–136, 2010.
 46. Malik G, Gorbounov N, Das S, Gurusamy N, Otani H, Maulik N, Goswami S, and Das DK. Ischemic preconditioning triggers nuclear translocation of thioredoxin and its interaction with Ref-1 potentiating a survival signal through the PI-3-kinase-Akt pathway. *Antioxid Redox Signal* 8: 2101–2109, 2006.
 47. Mattson MP. Hormesis defined. *Ageing Res Rev* 7: 1–7, 2008.
 48. McNeill DR and Wilson DM, 3rd. A dominant-negative form of the major human abasic endonuclease enhances cellular sensitivity to laboratory and clinical DNA-damaging agents. *Mol Cancer Res* 5: 61–70, 2007.
 49. Naganuma T, Nakayama T, Sato N, Fu Z, Yamaguchi M, Soma M, Aoi N, Usami R, Doba N, and Hinohara S. Haplotype-based case-control study between human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 gene and cerebral infarction. *Clin Biochem* 42: 1493–1499, 2009.
 50. Nakamura J and Swenberg JA. Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res* 59: 2522–2526, 1999.
 51. Narasimhan P, Sugawara T, Liu J, Hayashi T, Noshita N, and Chan PH. Overexpression of human copper/zinc-superoxide dismutase in transgenic animals attenuates the reduction of apurinic/apyrimidinic endonuclease expression in neurons after *in vitro* ischemia and after transient global cerebral ischemia. *J Neurochem* 93: 351–358, 2005.
 52. Ono Y, Furuta T, Ohmoto T, Akiyama K, and Seki S. Stable expression in rat glioma cells of sense and antisense nucleic acids to a human multifunctional DNA repair enzyme, APEX nuclease. *Mutat Res* 315: 55–63, 1994.
 53. Raffoul JJ, Heydari AR, and Hillman GG. DNA Repair and cancer therapy: targeting APE1/Ref-1 using dietary agents. *J Oncol* 2012: 370481, 2012.
 54. Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K, and Bonner W. Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev* 12: 162–169, 2002.
 55. Saito A, Maier CM, Narasimhan P, Nishi T, Song YS, Yu F, Liu J, Lee YS, Nito C, Kamada H, Dodd RL, Hsieh LB, Hassid B, Kim EE, Gonzalez M, and Chan PH. Oxidative stress and neuronal death/survival signaling in cerebral ischemia. *Mol Neurobiol* 31: 105–116, 2005.
 56. Singh S and Englander EW. Nuclear depletion of apurinic/apyrimidinic endonuclease 1 (Ape1/Ref-1) is an indicator of energy disruption in neurons. *Free Radic Biol Med* 53: 1782–1790, 2012.
 57. Stetler RA, Gao Y, Zukin RS, Vosler PS, Zhang L, Zhang F, Cao G, Bennett MV, and Chen J. Apurinic/apyrimidinic endonuclease APE1 is required for PACAP-induced neuroprotection against global cerebral ischemia. *PNAS* 107: 3204–3209, 2010.
 58. Tell G, Damante G, Caldwell D, and Kelley MR. The intracellular localization of APE1/Ref-1: more than a passive phenomenon? *Antioxid Redox Signal* 7: 367–384, 2005.
 59. Thompson JW, Narayanan SV, and Perez-Pinzon MA. Redox signaling pathways involved in neuronal ischemic preconditioning. *Curr Neuropharmacol* 10: 354–369, 2012.
 60. Tomkinson AE, Chen L, Dong Z, Leppard JB, Levin DS, Mackey ZB, and Motycka TA. Completion of base excision repair by mammalian DNA ligases. *Prog Nucleic Acid Res Mol Biol* 68: 151–164, 2001.
 61. Vasko MR, Guo C, and Kelley MR. The multifunctional DNA repair/redox enzyme Ape1/Ref-1 promotes survival of neurons after oxidative stress. *DNA Repair (Amst)* 4: 367–379, 2005.
 62. Vasko MR, Guo C, Thompson EL, and Kelley MR. The repair function of the multifunctional DNA repair/redox protein APE1 is neuroprotective after ionizing radiation. *DNA Repair (Amst)* 10: 942–952, 2011.
 63. Walker LJ, Robson CN, Black E, Gillespie D, and Hickson ID. Identification of residues in the human DNA repair enzyme HAP1 (Ref-1) that are essential for redox regulation of Jun DNA binding. *Mol Cell Biol* 13: 5370–5376, 1993.
 64. Wang G, Jiang X, Pu H, Zhang W, An C, Hu X, Liou AK, Leak RK, Gao Y, and Chen J. Scriptaid, a novel histone deacetylase inhibitor, protects against traumatic brain injury via modulation of PTEN and AKT pathway: scriptaid protects against TBI via AKT. *Neurotherapeutics* 10: 124–142, 2013.
 65. Williams MV, Wishnok JS, and Tannenbaum SR. Covalent adducts arising from the decomposition products of lipid hydroperoxides in the presence of cytochrome c. *Chem Res Toxicol* 20: 767–775, 2007.

66. Xu Y, Wang Y, Zheng Q, Li X, Li B, Jin Y, Sun X, and Sun G. Association of oxidative stress with arsenic methylation in chronic arsenic-exposed children and adults. *Toxicol Appl Pharmacol* 232: 142–149, 2008.
67. Zielinska A, Davies OT, Meldrum RA, and Hodges NJ. Direct visualization of repair of oxidative damage by OGG1 in the nuclei of live cells. *J Biochem Mol Toxicol* 25: 1–7, 2011.

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Abbreviations Used

8-OHdG = 8-hydroxy-2'-deoxyguanosine
 β -Pol = β -polymerase
 ANOVA = analysis of variance
 AP = apurinic/aprimidinic
 APE1 = apurinic/aprimidinic endonuclease 1
 APE1sc = scrambled shRNA sequence of APE1
 APE1t = shRNA sequence targeting APE1

ARP = aldehyde reactive probe
 ASF = area section frequency
 ATP = adenosine triphosphate
 BER = base-excision repair
 CMV = cytomegalovirus
 EDTA = ethylenediaminetetraacetic acid
 EEG = electroencephalography
 ELISA = enzyme-linked immunosorbent assay
 EtOH = ethyl alcohol
 fEPSP = field excitatory postsynaptic potential
 GFP = green fluorescent protein
 Gy = gray
 H2AX = histone H2A
 HA = hemagglutinin
 HPLC = high-performance liquid chromatography
 Hsp = heat shock protein
 I/O = input/output
 Lenti = lentivirus
 NF κ B = nuclear factor kappa-light-chain-enhancer of activated B cells
 OGG1 = 8-oxoguanine glycosylase
 PACAP = pituitary adenylate cyclase activating peptide
 PI = proton irradiation
 PSD-95 = postsynaptic density protein 95
 PUMA = p53 upregulated modulator of apoptosis
 SDS = sodium dodecyl sulfate
 shRNA = short hairpin RNA
 Sirt1 = sirtuin-1
 SSF = sampling section frequency
 SYP-1 = synaptophysin-1
 Tg-APE1 = transgenic rats overexpressing APE1
 tGCI = transient global cerebral ischemia
 TSF = thickness section frequency
 TUNEL = terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling
 WT = wild type
 XRCC = X-ray repair cross-complementing protein 1