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Base Excision Repair in the Mammalian Brain: Implication for Age related Neurodegeneration

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Abstract

The repair of damaged DNA is essential to maintain longevity of an organism. The brain is a matrix of different neural cell types including proliferative astrocytes and post-mitotic neurons. Post-mitotic DNA repair is a version of proliferative DNA repair, with a reduced number of available pathways and most of these attenuated. Base Excision Repair (BER) is one pathway that remains robust in neurons; it is this pathway that resolves the damage due to oxidative stress. This oxidative damage is an unavoidable byproduct of respiration, and considering the high metabolic activity of neurons this type of damage is particularly pertinent in the brain. The accumulation of oxidative DNA damage over time is a central aspect of the theory of aging and repair of such chronic damage is of the highest importance. We review research conducted in BER mouse models to clarify the role of this pathway in the neural system. The requirement for BER in proliferating cells also correlates with high levels of many of the BER enzymes in neurogenesis after DNA damage. However, the pathway is also necessary for normal neural maintenance as larger infarct volumes after ischemic stroke are seen in some glycosylase deficient animals. Further, the requirement for DNA polymerase β in post-mitotic BER is potentially more important than in proliferating cells due to reduced levels of replicative polymerases. The BER response may have particular relevance for the onset and progression of many neurodegenerative diseases associated with an increase in oxidative stress including Alzheimer's.

Keywords

BER; Neurodegeneration; DNA repair; Aging

Introduction

The brain is protected from many exogenous forms of DNA damage by the blood brain barrier. It is probable that the majority of the DNA damage encountered by the brain is the result of toxic byproducts of normal cellular respiration. This endogenous source of oxidative DNA damage may be particularly high in the brain, correlating with its large oxygen demands. The human brain consumes as much as 50% of the arterial oxygen provided by the lungs despite representing on average only 2% of the total weight of the body (Magistretti and Pellerin 1996). This oxygen is used to maintain a high metabolic rate required for the electrochemical transmissions between neurons and associated neural cells and also for continuous cell maintenance. The high metabolic rate inadvertently creates

reactive oxygen species (ROS), volatile molecules such as superoxide anions, hydroxyl radicals and hydrogen peroxide. These molecules are able to damage cellular components including genetic material in the nucleus and mitochondria, a fundamental aspect of the oxidative stress and mitochondrial theories of aging and the evolution of carcinogenesis. Considering the longevity of neurons coupled with their inability to replicate, continuous repair of DNA to maintain genomic integrity is essential for neuronal survival and normal neural function throughout the lifespan of an organism.

The DNA repair field has been dominated by research utilizing proliferating cells spurred primarily by investigators in the field of carcinogenesis. However, experiments conducted in post-mitotic cells and neural tissues have identified substantial differences in DNA repair between proliferating and non-proliferating cells ((Akbari et al. 2009;Wei and Englander 2008)). DNA repair in neurons appears to rely heavily on base excision repair (BER) but also utilizes non-homologous end joining (NHEJ) in the absence of homologous recombination. BER is the primary DNA repair pathway for the different, non-bulky forms of oxidative base modifications caused by ROS, as well as abasic sites and single-strand breaks that arise in DNA spontaneously, via the attack of ROS, or as intermediates during the repair response. The pathway is highly conserved amongst vertebrates and its importance can be extrapolated from the embryonic lethality of homozygous null murine models of many of the core BER enzymes (Figure 1). BER consists of three sub pathways: short patch BER (SP-BER) (Figure 2a), comprised primarily of enzymes dedicated exclusively to DNA repair; long patch repair (LP-BER) (Figure 2a), comprised of repair as well as replication proteins; and single stranded break repair (SSBR), an attenuated version of SP-BER that engages specialized termini “clean-up” proteins. SP-BER/SSBR are the major BER pathways in the brain, with residual LP-BER activity present in neuronal cells, yet at a reduced level in comparison to proliferating cells (Akbari et al. 2009;Wei and Englander 2008).

BER of an apurinic/apyrimidinic site (AP site) can be completed with as few as three BER enzymes, AP endonuclease (APE1), DNA polymerase β (Polβ) and DNA ligase (either I or III). Processing of a damaged base moiety requires the additional activity of one of a range of substrate specific DNA glycosylase enzymes. Despite the relative simplicity of BER compared to NHEJ, our knowledge of the pathways in the brain remains limited. The objective of this article is to provide a comprehensive analysis of BER from research conducted using rodent models and human brain tissue. From this work it is evident that BER is indispensable for neural development during embryogenesis and is critical for normal function throughout life. In addition, decline of BER with age may accelerate age related cognitive dysfunction and the progression of neurodegenerative disease.

DNA glycosylases

Despite the broad range of substrates recognized, DNA glycosylase activity during BER is restricted to the same two or three enzymatic events. Initially, the glycosylase recognizes and binds to the modified base. Each glycosylase has evolved to process a subset of base modifications. Often the glycosylase can also process other similar lesion conformations, giving the glycosylase group a high degree of substrate overlap not seen in the subsequent steps of BER. The large number of glycosylases, 11 in total in humans, emphasizes this notion, and bi functional glycosylases responsible for the excision of DNA modifications associated with oxidative stress are of particular interest in the brain.

Three glycosylase groups process oxidative base lesions: 8-oxoguanine DNA glycosylase 1 (OGG1), endonucleases VIII- like (Neil) and endonuclease III-like (NTH). The substrate overlap between the glycosylases hinders identification of individual glycosylase activity,

yet makes it possible to create viable homozygous null knockout mice. The phenotypes of these null animals are generally mild and only recently have researchers induced neural stress as a way of delineating the function of the glycosylases in the mammalian brain (Canugovi et al. 2012;Liu et al. 2011;Sejersted et al. 2011). Ogg1 null (*ogg1*−/−) mice were initially reported to only accumulate mutations after stress (Klungland et al. 1999). With this in consideration, it was proposed that OGG1 may be particularly important in the repair of oxidative lesions that rapidly accumulate after stroke, a neurological impairment with age association (Liu et al. 2011). Wild type (WT) and *ogg1*−/− mice were subjected to unilateral permanent middle cerebral artery occlusion (MCAO) and euthanized 48 hours after treatment. The *ogg1*−/− mice had larger infarct volumes compared to WT mice (summarized in Table 1), with deficient animals accumulating more stroke induced oxidative lesions (Liu et al. 2011) (Liu et al. 2011). This increase in damage impaired recovery after stroke was measured using the rota-rod test, revealing a significant decline in motor co-ordination after MCAO in the *ogg1*−/− mice (Liu et al. 2011). The *ogg1*−/− animals also performed more poorly in rota-rod testing before MCAO, suggesting a role for the glycosylase in protection from natural oxidative damage.

Similar stroke related work complementing the OGG1 study has recently been performed on two Neil mouse models. The Neil proteins cope with a broad range of oxidized pyrimidine and purine lesions (Hailer et al. 2005;Hu et al. 2005;Vik et al. 2012). In the case of Neil3, this protein preferentially repairs spiroiminodihydantoin and guanidinohydantoin which are oxidation products of the base lesions, 8-oxo-7,8-dihydroguanine (Liu et al. 2010). Sejersted *et al*. examined the effect of cerebral ischemia in a *neil3*−/− mouse model. As reported in the *ogg1*−/− mice, the *neil3*−/− mice had reduced neuronal regeneration and had greater remaining infarct volume 42 days after the insult (Sejersted et al. 2011). The authors observed a decline in the number of neural progenitor cells (DCX+) after treatment, correlating with *in vitro* work showing that *neil3*−/− neuroshperes grow poorly and exhibit an altered pattern of differentiation (Sejersted et al. 2011). Of note, unlike the previously described results in the *ogg1*−/− (Liu et al. 2011) mice, and in an independent *udg*−/− murine model (Endres et al. 2004), the amount of neural damage (infarct volume) after the ischemic treatment was not greater in the *neil3*−/− mice than sustained in the WT counterparts. This observation reiterates the different functions of glycosylases in various tissues, with *neil3*−/− potentially having a more prominent role in proliferating cells active in neurogenesis and neural regeneration after damage rather than DNA maintenance in post-mitotic neurons (Regnell et al. 2012).

In contrast, recent work investigating ischemic stroke-induced brain dysfunction in the *neil1*−/− mouse found that after a 48 hour recovery, the infarct volume was greater than in the WT control animals (Canugovi et al. 2012) (Table 1). The *neil1*−/− mice also had increased mortality, poorer recovery and enhanced stroke induced dysfunction in the motor system, consistent with the increase in tissue damage in the striatum and cortex of these mice. Based on these results, Neil1 appears to have a more prominent role in post-mitotic neuronal repair than Neil3, perhaps reflective of the protein substrate specificity or possible additional biochemical functions. The notion that alternate DNA glycosylases may have a cell specific role particularly in neuronal DNA repair potentially also affecting mitochondrial DNA repair is beyond the scope of this review but has been recently addressed by a number of review articles (Hegde et al. 2011;Hegde et al. 2012;Liu et al. 2012;Prakash et al. 2012). Of specific mention is that some glycosylases may preferentially repair lesions associated when associated with replication such as Neil3 which appears to be closely related to embryogenesis. However, in contrast Neil1 has been shown to be most active only after development ceases (Rolseth et al. 2008a). Hence, the investigators also evaluated whether the *neil1*−/− mouse had accelerated age related cognitive decline compared with middle (9–13 months) and old aged mice (30–33 months) (Canugovi et al.

2012). The *neil1*−/− deficient mice performed significantly worse at the Morris water maze at both middle and old age, although it could not be discerned whether the *neil1*−/−mice had accelerated age related neurodegeneration. Of note, there was no statistical difference in any of the other behavioral tests conducted including open field, rota-rod and fear conditioning, indicating that memory is particularly affected in these mice and there is not a systemic degeneration of the brain (Canugovi et al. 2012).

Overall, the results from the above studies reiterate that the single knockout glycosylase animals will display an overt neural phenotype when challenged with a stressor, such as cases of ischemic injury (summarized as Table 1). Under conditions of stress it appears to be predominantly proliferating cells that are most affected in the brain, with differentiation possibly being impacted, thus limiting the amount of neural stem cells available for repair of damage (Regnell et al. 2012). However, as reported in *neil3*−/− animals, there are certain glycosylase specific neural affects. Lack of Neil1 also leads to memory loss without induction of stress (Canugovi et al. 2012), and more studies are needed in the glycosylase deficient mouse models to clarify if this is a general effect of glycosylase deficiency. In comparison to *neil1* knockout mice, *nth1* mice had less oxidative lesion accumulation in the brain (Chan et al. 2009). A double *neil1/nth1* knockout mouse developed pulmonary and hepatic tumors at a far greater level then the single knockout animals suggesting that both oxidative glycosylases are required for genomic stability (Chan et al. 2009). Further studies are required to establish the long term effect of reduced glycosylase activity and clarify the individual roles of the glycosylases in the brain.

AP endonuclease

Despite the large variety of substrates that DNA glycosylases are able to recognize, the activity of the enzyme on these substrates “standardizes” the modifications by generating only a few types of intermediate repair products for further processing. This funneling effect, in conjunction with a lack of substrate overlap, increases the reliance of the genome on enzymes involved in subsequent steps of BER including APE1. APE1, also known as hAPE1, Apex and discovered independently as redox effector factor −1 (ref-1), works to process AP sites and a subset of obstructive 3’ termini, allowing for the subsequent action of Polβ. The early embryonic lethality of homozygous null *ape1*−/− mice (Xanthoudakis et al. 1996) (Figure 1) has hampered efforts to discern the neural function of the protein. Nevertheless, Raffoul *et al.* using a haploinsufficient mouse model (*ape1*+/−) measured *in vitro* G:U base mismatch repair activity in various tissues. Despite significant BER capacity differences in liver and testes, no difference was seen in the repair activity in brain extracts (Raffoul et al. 2004). Of note, the level of Polβ protein was reduced in the brain of *ape1*+/- mice, suggesting that the endonuclease is required for stable expression of the close BER partner, and perhaps indicating coupled regulation of the components of the BER pathway. Previous work from Rolseth and colleagues did report that levels of Neil1 protein showed strong expression in the hippocampus and neocortex. Interestingly, the level of Neil1 expression in the hippocampus increased as the mouse aged. This also correlated with an increase in fapy DNA glycoslyase activity in the hippocampus (Rolseth et al. 2008a;Rolseth et al. 2008b).

To date, there has been no behavioral or cognitive analysis to ascertain the impact of the *ape1* haploinsufficiency in the mouse model. Research has instead focused more on protein localization and regulation in the brain at various stages of development and after increased oxidative damage. As described previously for the glycosylases, the level of *ape1* (mRNA) correlated with areas of high cellular proliferation in the brain (Ono et al. 1995). Expression was high throughout the organ during embryonic development, but reduced in all regions post natal except the hippocampus, where mRNA levels remained high in the pyramidal and

granule cells at postnatal day 21. This early finding was supported by subsequent studies (Edwards et al. 1998a;Gillardon et al. 1997;Lewen et al. 2001;Walton et al. 1997) more focused on characterizing changes in APE1 protein levels after oxidative damage, while offering further insight into protein localization (information collated in Table 2). To summarize these studies, APE1 in the hippocampus can be induced after moderate DNA damage; this protective response is transient and does not occur for longer than 24–36 hours. The amount of DNA damage is also a determining factor for whether APE1 gets induced. Generally, the studies that report an induction of APE1 in the hippocampus do not observe apoptosis until after 48 hours, suggesting that the amount of DNA damage is not sufficient to induce an early apoptotic response. In contrast, when exposed to high levels of a DNA damaging agent that induces oxidative stress, the resulting immediate reduction of APE1 in the hippocampus is associated with rapid apoptosis within 24 hours, suggesting that the damage is overwhelming (Fujimura et al. 1999;Morita-Fujimura et al. 1999). This result also relates that the induction of APE1 is not part of early apoptotic signaling, but that the protein is increased due to its role in BER. The period of APE1 induction would likely correspond to the window of greatest therapeutic potential (presented graphically as Figure 3). Hence, APE1 levels 12 hours after damage may be an accurate predictor of the level of exposure and consequent tissue damage (Lewen et al. 2001). In support, APE1 has been shown to be neuroprotective after DNA damage (Jiang et al. 2008;Vasko et al. 2005;Vasko et al. 2011;Yang et al. 2010), with artificial induction of the protein leading to greater neural survival (Stetler et al. 2010).

The ability to mount a protective response against oxidative DNA damage may decline with age. To explore this hypothesis, young (3 month) and old (30 month) rats were exposed to isobaric hyperoxia, which elevates ROS levels in the brain and significantly increases apoptosis (Edwards et al. 1998b). Hyperoxia stimulated APE1 levels in the hippocampus and basal forebrain, but not in the cerebellum, of young animals after 6 hours. In contrast, aged mice had no increase in APE1 protein in the hippocampus, basal forebrain or cerebellum. Increasing treatment time did not induce any further increase in APE1 protein levels in the young animals, suggesting that the lack of induction observed in the older animals was not due to a delayed response. This research presents the interesting notion that the aged brain loses the ability to mount a protective response against DNA damage.

DNA polymerase β

Polβ is the principle BER polymerase in neurons. As a relatively small polymerase (39kDa), it lacks 3’ to 5’ exonuclease activity, with the proof reading function attributed to autonomous exonucleases such as APE1 (Hadi et al. 2002;Sukhanova et al. 2005) (Chou and Cheng 2002) or Werner (Harrigan et al. 2006) . Polβ is bi-functional in BER, possessing both a polymerization function and a 5’- dRP lyase activity, which has been identified as a rate limiting step in *in vitro* BER assays (Wang et al. 1997). In mammals, Polβ is found at high levels in the testis, where the polymerase has recently been shown to be critical for the development of mouse meiotic synapses, and also in the brain (Kidane et al. 2011;Kidane et al. 2010). Levels of *polβ* gene expression are high in the fetal brain, and in the adult hypothalamus and cerebellum (Su et al. 2004). The high levels of Polβ in the brain are consistent with it having a more prominent role in non-dividing cells (Sugo et al. 2000). Proliferating cells have the ability to use the LP-BER pathway, which can engage the replicative polymerases δ and ε (Figure 3b). Once a cell stops dividing, the levels of replicative components dramatically decrease, whereas levels of Polβ have been shown to increase or remain stable (Akbari et al. 2009;Wei and Englander 2008). Hence Polβ may become the primary DNA polymerase participating in SP-BER as well as LP-BER in non-replicating tissue (Figure 2b). In support of this repair capacity shift, LP-BER activity was measured in protein extracts from rapidly proliferating intestinal mucosa and compared to

extracts from the post-mitotic dentate gyrus hippocampal brain region (Wei and Englander 2008). LP-BER activity was substantially reduced in the hippocampal tissue suggesting that the repair of LP-BER substrates may be specifically impaired in the brain. Further, in the post-mitotic dentate gyrus, Polβ was involved in an alternate PCNA independent LP-BER activity that did not utilize the replicative polymerases. The important role of Polβ in the brain is also corroborated by the prominence of the polymerase in isolated neuronal and astroglial cultures (Raji et al. 2002). Using various oligonucleotide substrates including a one nucleotide gap and a nick substrate, we have evidence that the polymerase incorporation step of SP-BER is rate limiting in differentiated SH-SY5Y neuroblastoma cells (work in progress). However, both polymerase activity and ligase activity are substantially reduced in post-mitotic (differentiated) neurons when compared to proliferating (undifferentiated) neuroblasts, and both activities are needed to induce DNA repair to undifferentiated levels (Sykora et al., manuscript submitted) . The need for both polymerization and ligation activities to induced BER activity is corroborated by research using isolated neuronal cultures derived from the rat brain (Swain and Rao 2012).

The *pol*β homozygous mouse (*pol*β−/−) further supports the important role of the protein in post-mitotic tissue (Sugo et al. 2007). Unlike the early embryonic lethality reported after knockout of other core BER genes, *polβ(−/−)* embryos develop to term (Figure 1)(Sugo et al. 2000). While the *polβ(−/−)* mice are at predicted Mendelian ratios up to embryonic day (E) 18.5, they are smaller than litter mates and at birth do not induce a breathing response. The late stage developmental death occurred due to problems associated with neural differentiation, further evidence that the protein is particularly important in post-mitotic tissue. Sugo et al. reported apoptosis in the developing nervous system at E12.5, including in the cortex, dorsal root ganglion and the hindbrain (postulated to be the cause of the respiratory defect)(Sugo et al. 2000). Cell death in these brain regions is associated with p53 activation, suggesting that DNA damage is responsible for the apoptotic response. The *p53 (−/−) pol*β (−/−) animals had less neural apoptosis, yet retained the neural abnormalities of the *pol*β (−/−) mice and did not survive after birth (Sugo et al. 2004). Importantly, no histological abnormalities were observed in any other *pol*β (−/−) mouse organ (Sugo et al. 2000).

Associated studies using a *parp* (−/−) *pol*β (−/−) mouse resulted in a more severe neural phenotype (Sugo et al. 2007) seen also with a DNA-PKcs (−/−) *pol*β (−/−) mouse (Niimi et al. 2005). An assessment of mutational frequency found that the *pol*β (−/−) animals actually had lower levels of mutation in the brain then WT litter mates, proposed to be the result of increased apoptosis that clears damaged neurons (Niimi et al. 2006). Recently, a mouse carrying a *pol*β homozygous mutation (Y265C, known to cause gastric carcinoma) has been established; this mouse has reduced cellular DNA polymerase activity but wild type dR lyase activity. The mutant animals are born at Mendelian frequency, but only 40% survive after birth. This result suggests that both activities of the enzyme are required for normal neural development (Senejani et al. 2012).

Neural studies investigating age related changes in Polβ have been limited. Brain samples from aged mice (22–24 month) had significantly reduced *in vitro* DNA repair activity when compared to young animals (4–6 months) (Cabelof et al. 2002). Consistent with this decrease, activity on a gap filling substrate, used to measure Polβ activity, was reduced to comparable levels, and western results showed a decrease in Polβ protein levels to approximately 50% of the levels seen in young animals. These findings have been supported by a decline in DNA repair activity in protein extracts from neurons taken from old rats (Rao et al. 2001;Rao et al. 2000), a feature that can be restored to normal by supplementation with purified Polβ. It is unclear whether a sustained reduction in Polβ has an impact on behavioral parameters.

DNA ligase

The re-sealing of the DNA phosphodiester backbone represents the final step in BER and is conducted by the ligase class of enzymes. There are currently two well characterized mammalian BER ligases, I (Lig1) and III (Lig3), with Lig3 working in complex with the scaffold protein XRCC1 *in vivo*. Homozygous null knockdowns of either Lig1 or Lig3 result in severe systemic developmental defects and embryonic lethality (Figure 1), indicating that despite some functional redundancy between the two ligases at a cellular level, both are needed for normal embryonic development. High levels of ligase activity were detected in the cerebellum of developing rats, peaking at 5 days after birth and only declining to levels in the rest of the brain after 20 days (Nakaya et al. 1977). This increase correlates with a period of rapid growth in the cerebellum, further evidence of the importance of BER in proliferating regions of the brain and during neural development (Nakaya et al. 1977). Moreover, *lig1* gene expression is highest in proliferating cells, although reduced levels are present in rat neurons suggesting that Lig3 cannot substitute for all of Lig1 related activities (Montecucco et al. 1992). In support, immunodepletion of Lig1 inhibited LP-BER *in vitro* (Sleeth et al. 2004).

Recently, Lig3 Nes-Cre ($Lig3^{\text{Nes-cre}}$) mice were created with a conditional neural specific inactivation of Lig3 to determine the role of the ligase throughout the nervous system. These mice were born alive, although they became growth retarded and ataxic, dying before 20 days of age (Gao et al. 2011). Macro analysis revealed a smaller brain in the mutant animals; cerebellar volume was particularly affected displaying reduced proliferation and increased apoptosis of granule neuron progenitors. Despite the close association with Lig3 *in vivo*, XRCC1 Nestin-Cre ($XRCC1^{\text{Nes-Cre}}$) mice did not show a similar neural physiology. Using a mitochondrial marker, it was determined that the $Lig3^{\text{Nes-cre}}$ mice had mitochondrial dysfunction not observed in WT or $XRCC1^{\text{Nes-Cre}}$ derived astrocytes. By comparing the toxicity of DNA damaging agents in astrocytes derived from the WT, $XRCC1^{\text{Nes-Cre}}$, or $Lig3^{\text{Nes-cre}}$ mice, it became clear that Lig3 was not critical for nuclear BER in the brain, but for mitochondrial BER (Gao et al. 2011). The main conclusion drawn from the study is that in quiescent cells the major nuclear BER ligation activity comes from Lig1. Analysis of aged tissue supports this conclusion, with an *in vitro* study conducted on rat cortical neurons reporting an age dependent decrease in total ligase activity (Swain and Rao 2012). A separate study found no age related decline of Lig3 in the murine brain (Imam et al. 2006), suggesting that the reduction in ligase activity is due to a decrease in Lig1 protein levels. This correlated with Lig1 contributing the majority of ligation activity (Sleeth et al. 2004), with a decrease reported after cellular senescence (Akbari et al. 2009).

There are no reports investigating the neural phenotype of heterozygous Lig3 or Lig1 mice. However, a comprehensive study of the XRCC1 heterozygous (*xrcc1 -/+*) mice did not show an accelerated aging phenotype, and the mice had normal histology of the brain at 18.5 months old (McNeill et al. 2011). Behavioral studies, including testing of spatial memory using the Morris water maze, also indicated no difference in memory or learning ability. While motor coordination and overall activity were deemed normal in the *xrcc1 -/+* mice, it is noteworthy that a small number of animals exhibited behavioral features commonly associated with stroke, namely impaired lower leg movement or tilting of the head. This observation is consistent with the emerging evidence that BER can modulate the cerebral response to ischemia and the risk of stroke in rodents and humans. While there are as yet no reports of any human neurodegenerative disorders specifically caused by mutations in the ligase or *XRCC1* genes, there exist three rare autosomal recessive neurological disorders that likely stem from a defect in SSBR: ataxia with ocular motor apraxia and spinocerebellar ataxia-1 (SCAN1), ataxia with oculomotor apraxia type1 (AOA1), and microcephaly, infantile-onset seizures, and developmental delay (MCSZ) (Ahel et al. 2006;El-Khamisy et

al. 2005). Presently, it is unclear whether the strong neural phenotypes of these disorders arise from a breakdown in nuclear and/or mitochondrial BER (Becherel et al. 2006;Harris et al. 2009;Sykora et al. 2011)

Mitochondrial BER

Increased reactive oxygen species are generally produced due to inefficiencies in mitochondrial bioenergetics. The mitochondrial DNA (mtDNA) is close to the site of ROS production, and oxidative stress represents the major source of DNA mutation in this organelle. To compensate for this damage the organelle also uses the BER system, and recent work has significantly enhanced our understanding of the pathway in mitochondria. Initially mitochondrial BER (mtBER) was believed to be only a subset of nuclear BER (nBER), but we now know that mitochondria do contain all three of the nBER sub pathways with some differences (protein involvement summarized as Table 3). All proteins involved in mtBER are encoded by the nuclear genome with many mitochondrial specific analogs of nBER proteins. While many of the BER proteins are available to both compartments, current research suggests that the importance of particular BER proteins can be different between the nucleus and mitochondria. This may be due to alternate backup repair pathways in the nucleus. Perhaps the clearest evidence of this difference is reported with Lig3. This ligase can be used in the nucleus and in the mitochondria, however, as discussed earlier it has a dominant role in mitochondrial DNA repair and replication. The phenotype of this animal includes severe cerebellar atrophy with ataxia, and importantly this phenotype is not seen in *XRCC1*$^{(nes\text{-}cre)}$ animals, indicating that the phenotype is indeed due to a breakdown of mtDNA maintenance.

There are close similarities in the phenotype of the *Lig3*$^{(nes\text{-}cre)}$ mouse with a specific set of human disorders associated with the breakdown of SSBR termini modifying proteins TDP1 and aprataxin. As with Lig3, both TDP1 and aprataxin has been identified in both the nucleus and mitochondria, and these proteins may also have prominent roles in the mitochondria. In support, aprataxin deficient cells accumulate more mtDNA damage then measured in the nucleus (Sykora et al. 2012). We have also observed more TDP1 in mitochondrial protein extracts then in total protein extracts (Figure 3), generally agreeing with other published results(Das et al. 2010a) implying that this protein has a more prominent role in mtDNA repair. Furthermore, PNKP, another specialized SSBR enzyme, was recently discovered in the mitochondria as well(Tahbaz et al. 2012a). However, human neural symptoms associated with PNKP mutation do not correspond to those seen with *Lig3*$^{(nes\text{-}cre)}$ or human aprataxin or TDP1 deficiency, suggesting that in this case, the SSBR protein may not have a predominantly mitochondrial role (Sejersted et al. 2011).

BER and neurodegeneration

There is a growing body of literature linking oxidative DNA damage to age related neurodegeneration and neurodegenerative diseases. The key role BER plays in the resolution of oxidative damage ensures that the pathway is maintained in post-mitotic neurons and also in proliferating neural progenitors. In cell culture, the burden of oxidative stress elicits a short term protective response including a transient increase in BER protein and activity levels. This appears to correlate with some of the *in vivo* results described within after stress. The notion that the aging brain may have a reduced protective response to DNA damage has serious implications in human neurodegenerative disorders (Edwards et al. 1998b). However, BER studies conducted in AD samples appears not to support this hypothesis. A post mortem study comparing APE1 levels in normal and AD brains sections found high immunoreactivity in the AD hippocampus and in the adjacent temporal cortex compared to control samples. Further, the distribution of APE1 positive cells correlated with

the presence of neural injury. The authors also identified a correlation between regions of high APE1 and senile plaques, however, a quantitative analysis was not undertaken to confirm this observation (Tan et al. 1998). In a similar study, APE1 expression in the AD cortex again clearly showed an increase in APE1 positive cells in AD biopsies when compared to controls ((Marcon et al. 2009) and also in (Weissman et al. 2007)). Furthermore, cultured neurons exposed to β-amyloid fragments, a hallmark of AD, re-enter cell cycle though ending in apoptotic cell death (Copani et al. 2002). The β-amyloid related death was linked to Polβ over-expression, with replication initiated by Polβ, independent of other BER co-factors (Copani et al. 2006). In support, AD autopsy brain specimens showed a correlation between severity of pathology and increased Polβ expression between Braak stage 0 and III/IV in cortical samples (Copani et al. 2006). Protein expression declined in later stages potentially due to substantial neuronal loss. This late stage decline in Polβ is also supported by evidence that BER activities and Polβ protein levels were substantially decreased in both the cerebellum and intra perennial lobes of late stage AD (Braak stage 6) compared to controls (Weissman et al. 2007). Similarly, there was a decrease in Polβ initiated gap filling activity and uracil incision correlating with Braak stage. What we can deduce from the reports is that the AD brain does appear to retain the ability to mount a protective response until late stage progression of the disease.

Closing remarks

What is clear from the studies reviewed here is that there are many factors which influence BER protein levels in the brain. These have made collation of results more difficult and severely limited the conclusion that could be drawn from the studies as a whole. However, with that in consideration, it is clear that in general BER is at its highest level during neurogenesis in the brain. This may be due to the particularly high oxidative load during this phase of development and/or correlated with the genomes replication. The influence of BER in the aging brain is less understood, however, accumulating evidence suggests that it cannot be underestimated in the progress of neurodegeneration.

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Highlights

1. APE1 protein levels in the brain can be used to predict neural damage.
2. DNA polymerase β becomes more important in post mitotic neuronal cells.
3. Ligase 1 is the primary BER ligation enzyme in the nucleus of post mitotic cells.
4. Ligase 3 participates largely in mitochondrial repair and replication.
5. BER enzymes are at highest level during embryogenesis and declining with age.

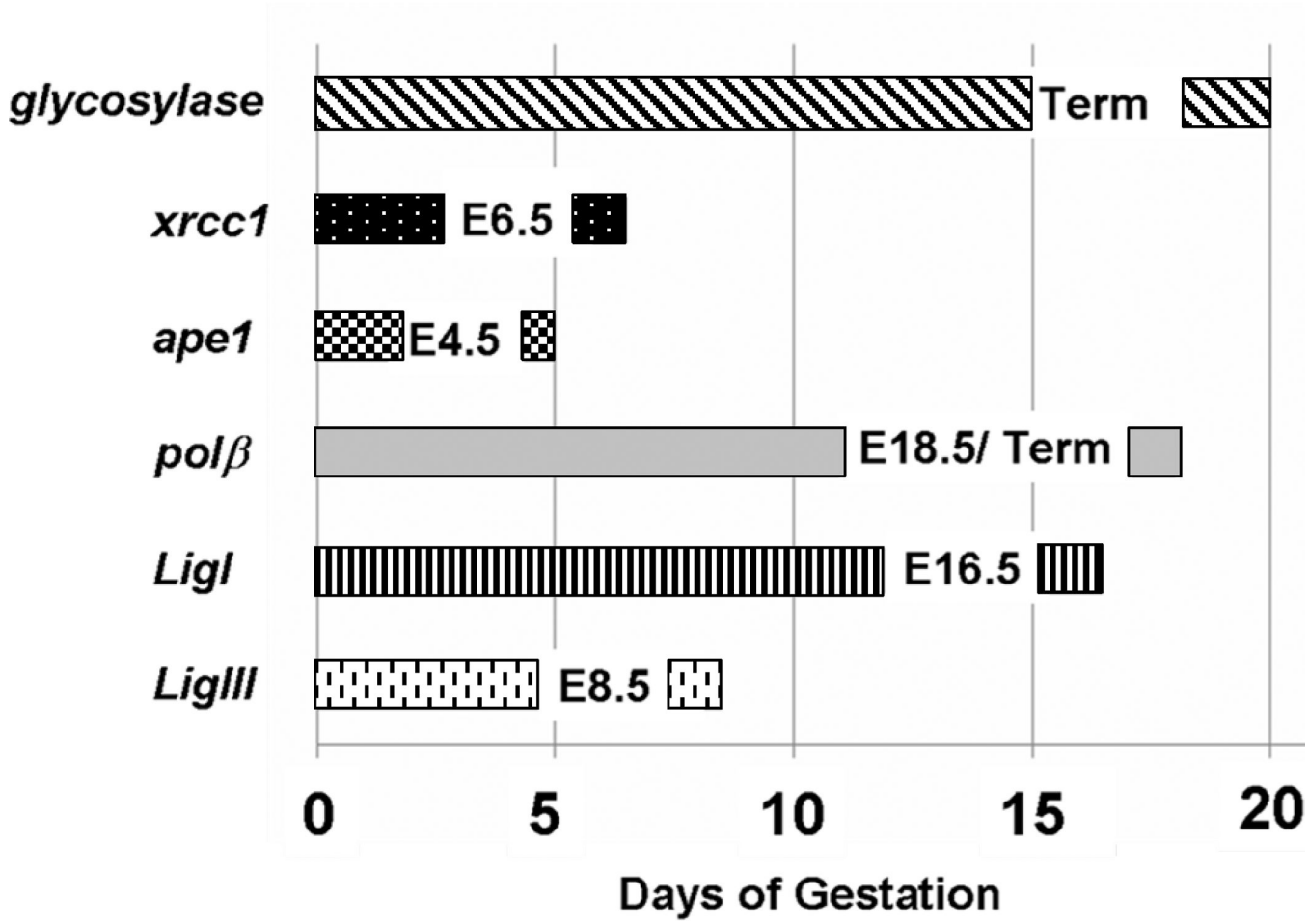

Figure 1. Embryonic lethality of homozygous knockout mice

Only mice null for glycosylases are viable. All core BER enzymes are embryonically lethal. *polβ* mice may develop to term but are smaller than wild type littermates and have no induction of breathing response with death occurring shortly after birth (E= embryonic day).

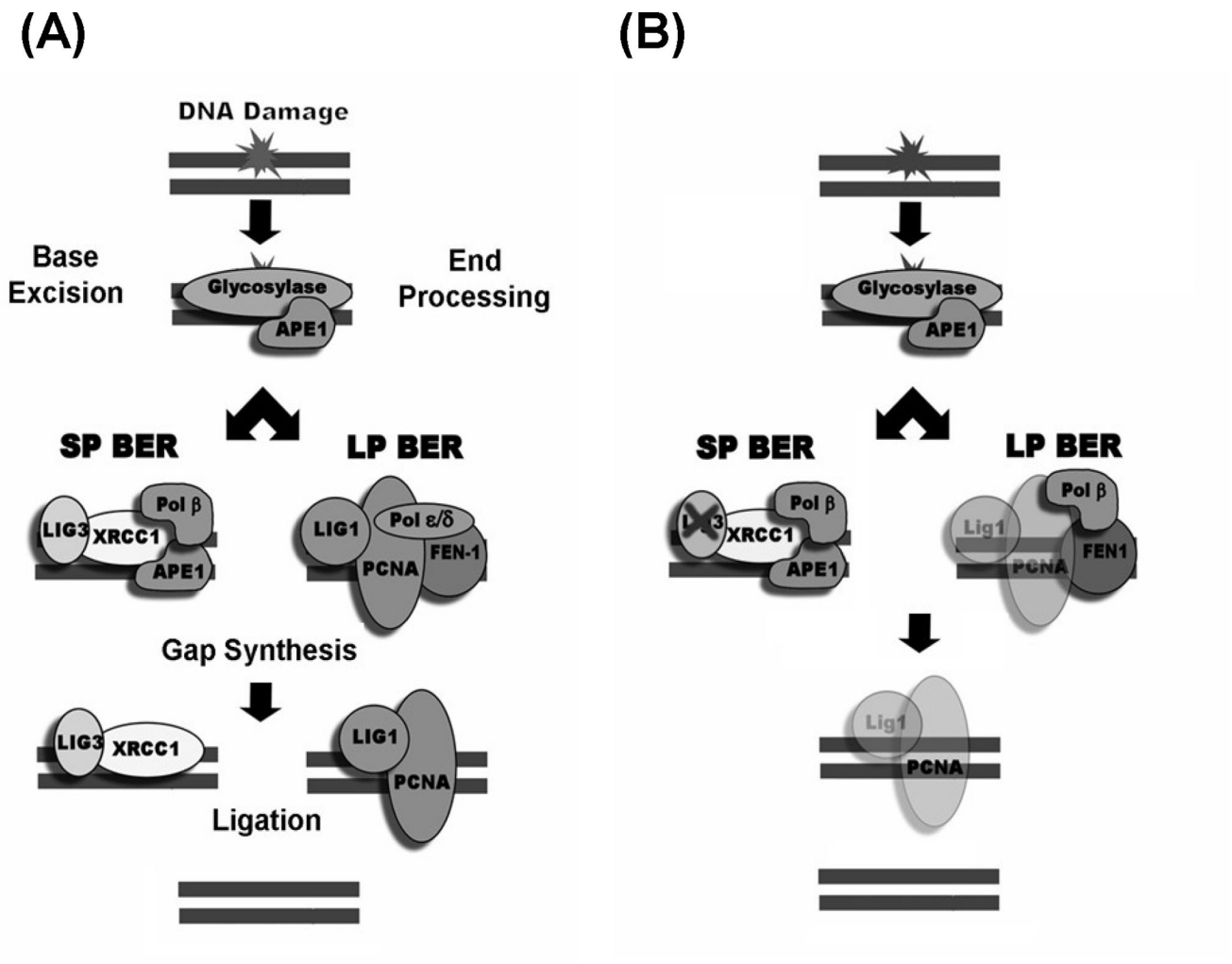

Figure 2.

(A) Diagrammatic representation of Base Excision Repair (BER) The initial step of BER use the activity of the glycosylase enzymes and AP endonuclease 1 (APE1), to excise damage nucleotides and processes modified termini. The pathway can then proceed through one of two sub pathways (short patch (SP-BER)) or (long patch (LP-BER)). SP-BER then uses the activity of DNA polymerase Beta (Polβ) to re-insert the correct nucleotide and DNA ligase 3 (Lig3)/XRCC1 to Reseal the phosphodiester backbone. In contrast LP-BER uses protein involved in replication including DNA polymerase delta and epsilon (Pol δ/ε) in conjunction with Flap endonuclease 1 (FEN-1) and proliferating cell nuclear antigen (PCNA) providing scaffolding for the complex . DNA ligase 1 (Lig1) provides the ligation activity. (B) Base excision repair in post-mitotic neurons. There is accumulating evidence that the repair pathway changes substantially after neural differentiation. Polβ takes a more prominent role after differentiation participating in both SP and LP-BER in the absence of the replicative polymerases. Lig3 does not participate in nuclear BER in post-mitotic cells. Ligation appears to be heavily dependent on Lig1 despite reduced levels of the protein in non-proliferating cells.

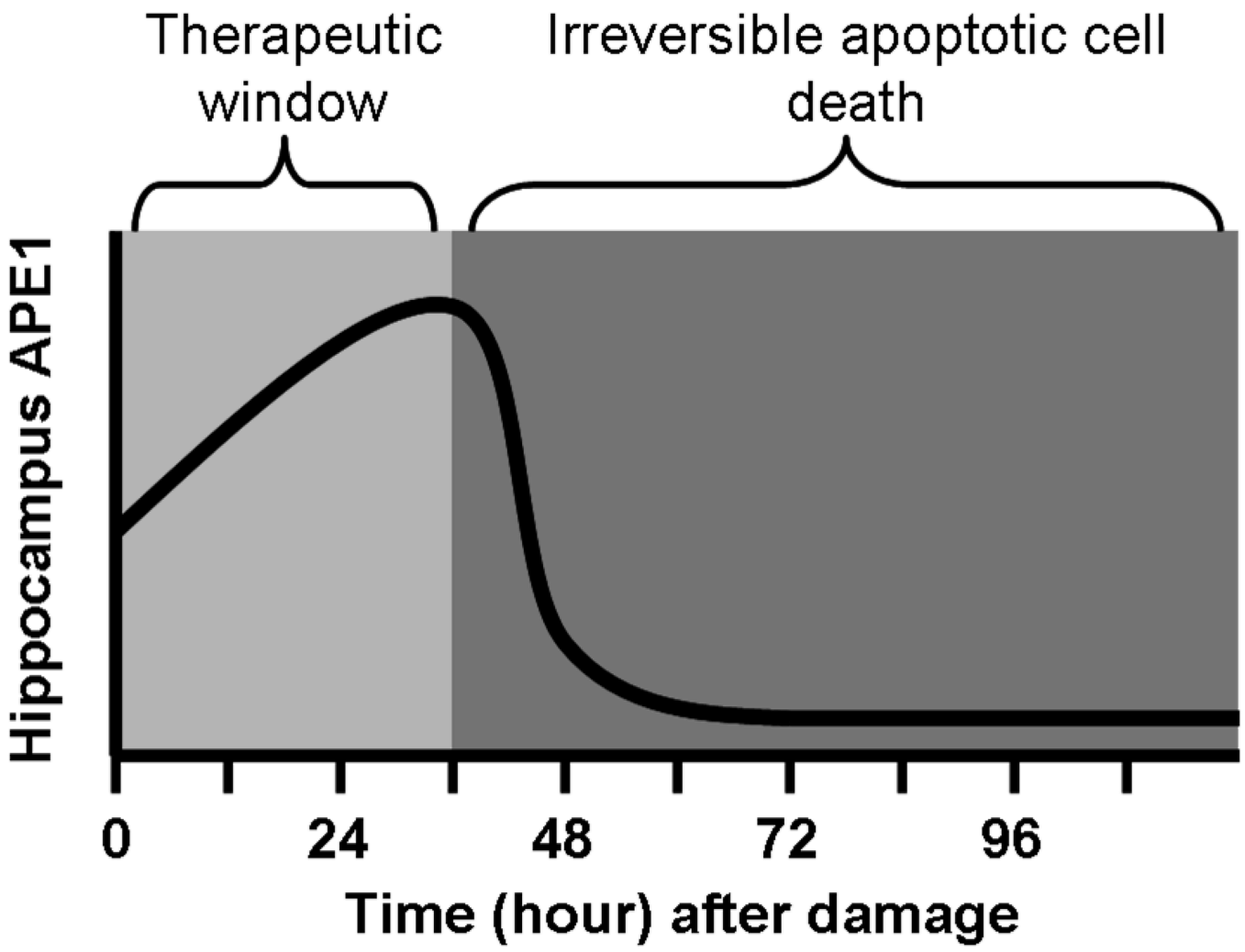

Figure 3. Graphical summary of APE1 levels in the hippocampus after moderate DNA damage

From studies conducted after damage (summarized Table 2) we can estimate that the therapeutic window after brain injury corresponds with the level of APE1. This window does not extend beyond 24 hours and is predicted to be part of a protective response against elevated DNA damage. After this time, cells with high levels of APE1 are observed to go into apoptotic cell death and are unlikely to respond to any therapeutic intervention.

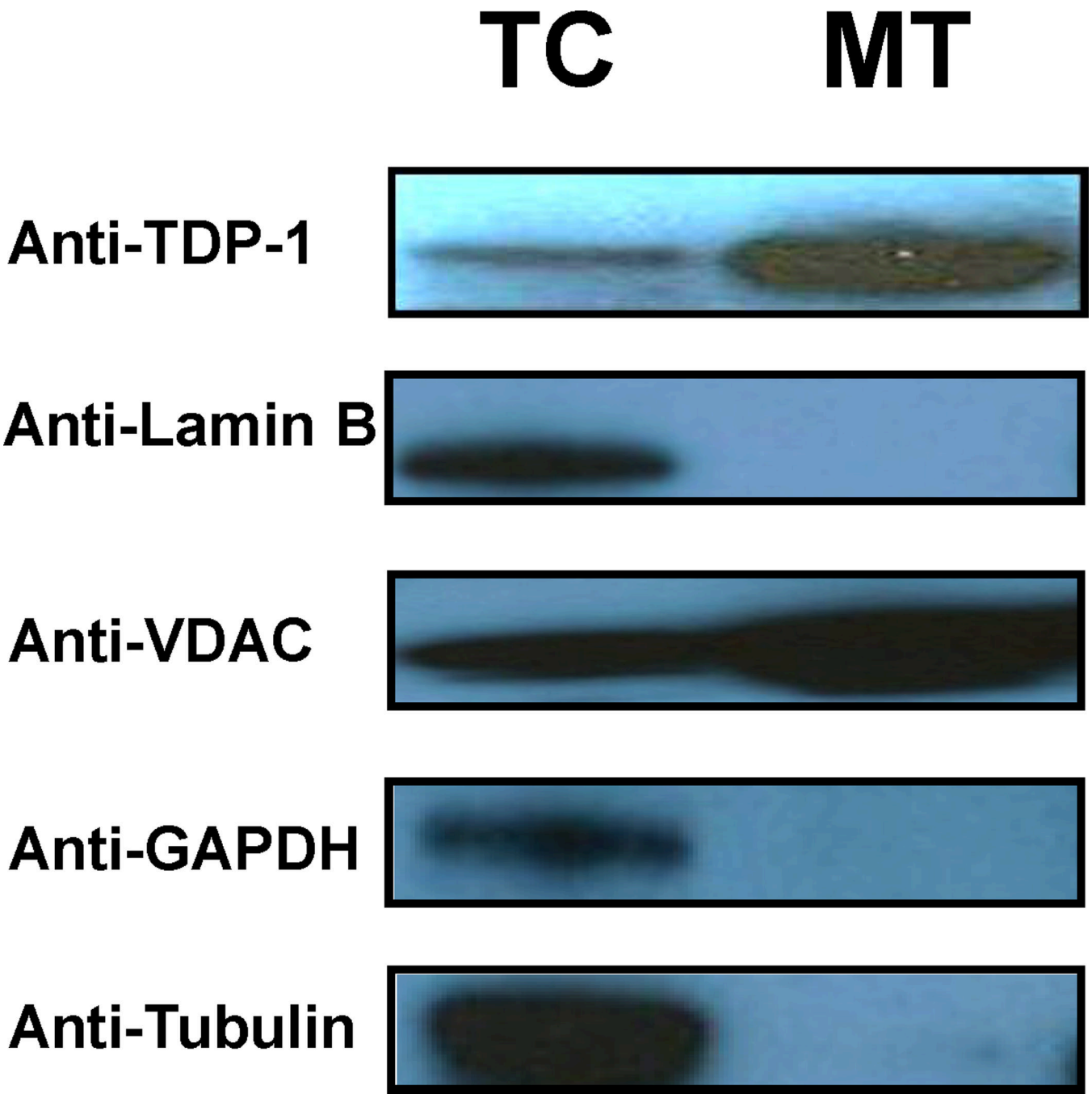

Figure 4. Mitochondrial TDP1 protein

20 μg of total cellular (TC) or Mitochondrial (MT) protein extracted from SH-SY5Y neuroblastoma cells was used in western analysis to determine the level of TDP1 protein expression. High levels of TDP1 were detected in the mitochondrial extract in comparison to the total cellular extract. Lamin B antibody was used to detect nuclear contamination in the mitochondrial fraction. VDAC antibody showed mitochondrial enrichment in the mitochondrial fraction. Antibodies to GAPDH and Tubulin were used to determine the level of cytoplasmic contamination.

Table 1

Glycosylase Homozygous Mice Post Treatment.

Mouse	Treatment	Infract Volume vs. WT	Recovery	Behavioral Differences vs. WT	Reference
ogg1 (−/−)	MACO	Larger	Impaired	Decline in Motor Coordination	(Liu et al. 2011)
neil1 (−/−)	MACO	Larger	Impaired	Decline in Motor Coordination	(Canugovi et al. 2012)
neil3 (−/−)	CCA	No Difference	Impaired	Not Assessed	(Sejersted et al. 2011)

Treatment definitions: MACO (Middle Cerebral Artery Inclusion), CCA (Common Cartoid Artery inclusion)

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Table 2

APE1 Hippocampal protein levels after oxidative injury

Animal	Age	Treatment (Duration)	Recovery (hrs)	APE Level Hippocampus	Reference
Rat	21 Day	Ischemia (1 hr)	24	Increase (NS)	(Walton et al. 1997)
		Hypoxia (15min)	48	Decrease	
			72	Decrease	
			168	Decrease	
Rat	3 month	MACO (1hr)	6	Decrease	(Edwards et al. 1998a)
Rat	3 month	Hyperoxia			
		(6hr)	0	Increase	(Edwards et al 1998b)
		(12hr)	0	No change	
		(24hr)		Increase (NS)	
		(48hr)		No change	
Rat	Not stated	Cardiac arrest (10min)	6	Increase	(Gillardon et al. 1997)
			24	Increase	
			72	Decrease	
Mouse	Not stated	Trauma	1	Decrease	(Lewen et al. 2001) *
			4	Decrease	
			24	Decrease	
Rat	Not stated	CCA (10 min)	48	Decrease	(Kawase et al. 1999)
			72	Decrease	

Abbreviations: MACO (Middle Cerebral Artery Inclusion), CCA (Common Cartoid Artery inclusion), NS (Not significant),

* early induction of apoptosis.

Table 3

Proteins involved in nuclear and mitochondrial BER

Protein	Nuclear	Mitochondrial	Reference*
OGG1	Yes	Yes	(de Souza-Pinto et al. 2001)
Neil1	Yes	Yes	(Hu et al. 2005)
Neil3	Yes	No Data	No Data
UNG	Yes	Yes	(Akbari et al. 2007)
NTH1	Yes	Yes	(Takao et al. 1998)
APE1	Yes	Yes	(Mitra et al. 2007)
Polβ	Yes	No	(Hansen et al. 2006)
Polγ	No	Yes	(Bertazzoni et al. 1977)
XRCC1	Yes	No	(Lakshmipathy and Campbell 2000)
TFAM	No	Yes	(Fisher and Clayton 1988)
Lig3	Yes	Yes	(Lakshmipathy and Campbell 1999)
Lig1	Yes	No#	(Donahue et al. 2001)
APTX	Yes	Yes	(Sykora et al. 2012)
TDP1	Yes	Yes	(Das et al. 2010)
PNKP	Yes	Yes	(Tahbaz et al. 2012)

Refer to text for all abbreviations,

*references for mitochondrial localization,

#*protein is found in prokaryotic mitochondria but not in eukaryotic*.