

REVIEW

DNA-dependent protein kinase and DNA repair: relevance to Alzheimer's disease

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Abstract

The pathological hallmark of Alzheimer's disease (AD), the leading cause of senile dementia, involves region-specific neuronal death and an accumulation of neuronal and extracellular lesions termed neurofibrillary tangles and senile plaques, respectively. One of the biochemical abnormalities observed in AD is reduced DNA end-joining activity. The reduced capacity of post-mitotic neurons for some types of DNA repair is further compromised by aging. The predominant mechanism to repair double-strand DNA (dsDNA) breaks (DSB) is non-homologous end joining (NHEJ), which requires DNA-dependent protein kinase (DNA-PK) activity. DNA-PK is a holoenzyme comprising the p460 kDa DNA-PK catalytic subunit (DNA-PK_{cs}) and the Ku heterodimer consisting of p86 (Ku 80) and p70 (Ku 70) subunits. Ku binds to DNA ends first and then recruits DNA-PK_{cs} during NHEJ. However, in AD brains, reduced NHEJ activity has been reported along with reduced levels of DNA-PK_{cs} and the Ku proteins, indicating a potential link between AD and dsDNA damage. Since age-matched control brains also show a reduction in these protein levels, whether there is a direct link between NHEJ ability and AD remains unknown. Possible mechanisms involving the role of DNA-PK in neurodegeneration, a benchmark of AD, are the focus of this review.

Introduction

DNA, the hereditary material of all living organisms, is sensitive to damages from oxidation, hydrolysis, and methylation. The living cells are equipped with the ability for efficient DNA repair systems, such as repair base damage (base excision repair, or BER), nucleotide damage (nucleotide excision repair, or NER), single-strand breaks

(single-strand break repair), and double-strand breaks (double-strand break repair). Double-strand DNA (DSB) breaks are considered the most lethal form of DNA damage. In eukaryotes, there are two major DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is the predominant pathway in higher eukaryotes and is active throughout the cell cycle [1-3], whereas HR is generally limited to S and G₂ when a sister chromatid is available as a repair template [4]. The primary role of NHEJ is to resolve DNA double-strand breaks, and data implicate DNA-dependent protein kinase (DNA-PK) as a central regulator of DNA end access [5].

Alzheimer's disease (AD) is a central nervous system neurodegenerative disease. The pathological presentation of AD, the leading cause of senile dementia, involves regionalized neuronal death and an accumulation of neuronal and extracellular lesions termed neurofibrillary tangles and senile plaques, respectively (reviewed in [6]). Several independent hypotheses have been proposed to link the pathological lesions and neuronal cytopathology with, among others, apolipoprotein E genotype [7,8], hyperphosphorylation of cytoskeletal proteins (neurofilaments and Tau) [9], and amyloid- β metabolism [10]. However, not one of these theories alone is sufficient to explain the diversity of biochemical and pathological abnormalities of AD. There is limited evidence for neuronal loss in most amyloid precursor protein (APP) models, and when neuronal loss was noted, it was modest [11-13].

Cellular damage by oxidative stress has been proposed as a causative factor in pathophysiology of AD and normal aging. Elevated levels of oxidative damage in both nuclear DNA and mitochondrial DNA have been reported in brains of patients with AD [14], and BER deficiency has been found in post-mortem brains of sporadic patients with AD [15]. However, impaired BER activity found in neuropathological brain regions and in the cerebellum where there is no neuronal death indicates that BER deficiency is not specific to human AD brains [16]. The lack of a difference in BER activity between wild-type and AD model mice brains in any age group [17] indicates that species-specific mechanisms may be

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involved in AD progression. Nonetheless, progressive neuronal loss due to cumulative damage to DNA and lack of DNA repair has been hypothesized to contribute to AD and stroke [18,19]. Moreover, human hereditary syndromes with genetic defects in the DNA repair process manifest in early-onset developmental and progressive neurodegeneration, indicating that defects in DNA damage repair are neuropathological [20,21].

In the four major DNA repair pathways (double-strand DNA (dsDNA) break repair (HR and NHEJ), NER, BER, and mismatch repair), key proteins, including some with dual functions, participate in DNA damage sensing/repair and apoptosis (Table 1) [16]. The focus of this review is on how DNA-PK activity may be linked to AD and whether a 'cause and effect' scenario emerges from the reported studies.

DNA-dependent protein kinase, a multi-subunit enzyme

DNA-PK is a PI3 kinase family member, and like targets of other members (ATR and ATM) of this family, its preferential targets of phosphorylation are the serines and threonines followed by a glutamine (S-T/Q sites), although other S-T/hydrophobic residues are also phosphorylated [22]. DNA-PK enzyme activity is essential for NHEJ [5]. Although DNA-PK is implicated in a variety of functions from activation of innate immunity [23] to regulation of gene expression [24], its primary cellular function is to initiate NHEJ. A multi-subunit enzyme, DNA-PK consists of a catalytic subunit (DNA-PK_{cs}), p460, and a regulatory subunit called Ku. The Ku protein is a heterodimer composed of 70-kDa (Ku70) and 80-kDa (Ku80) subunits and has the capability of binding selectively to specific forms of DNA [25,26]. In this capacity, it functions as the regulator of DNA-PK that is active in transcription, DNA recombination, and DNA repair [27-29]. Its role in DNA repair was not formally proven until the emergence of studies implicating Ku as the defective factor in cells hypersensitive to DNA-damaging agents [30].

DNA-dependent protein kinase activators

Ku binds DNA ends in a sequence-independent manner, and in the absence of DNA-PK_{cs}, the extreme DNA terminus is bound in an accessible channel [31]. Ku has strong avidity for DNA with a variety of end structures, such as blunt, over-hanged, hair-pinned, and damaged. Ku can also recognize gaps and nicks in dsDNA, indicating possible roles of DNA-PK in the repair of damage other than DSBs [32], and is particularly suited to do this since DNA-PK_{cs} assembles onto Ku-bound DNA regardless of end structure [33]. Although DNA-PK_{cs} has innate affinity (itself) for DNA ends (in low salt conditions), Ku is required for targeting DNA-PK_{cs} to damaged DNA in

Table 1. Key proteins involved in various types of DNA repair

DNA repair type	Key proteins involved
BER	Ref-1/Ape, PARP-1, and p53
NER	XPB, XPD, p53, and p33 (ING1b)
MMR	MSH2, MSH6, MLH1, and PMS2
HR	BRCA1, ATM, ATR, WRN, BLM, Tip60, and p53
NHEJ	DNA-PK

ATM, Ataxia telangiectasia mutated protein; ATR, Ataxia telangiectasia and Rad3-related protein; BER, base excision repair; BLM, Bloom's syndrome gene product; BRCA1, breast cancer 1, early onset gene product; DNA-PK, DNA-dependent protein kinase; HR, homologous recombination; ING1b, inhibitor of growth protein 1 gene product; MLH1, MutL homolog 1, colon cancer, non-polyposis type 2 (*Escherichia coli*); MMR, mismatch repair; MSH2, MutS homolog, colon cancer, non-polyposis type 1 gene product; MSH6, MutS homolog 6 gene product; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NFT, neurofibrillary tangle; PARP-1, poly (ADP-ribose) polymerase 1; PMS2, post-meiotic segregation increased 2 gene product; Ref-1/Ape, DNA-(apurinic or apyrimidinic site) lyase; Tip60, Tat interactive protein; WRN, Werner syndrome gene product; XPB, Xeroderma pigmentosum B gene product; XPD, Xeroderma pigmentosum factor D.

physiologic conditions and in living cells [34]. Although any DSB discontinuity can activate DNA-PK, its activation varies considerably depending on the end structure, and studies show kinase activation in *trans* (achieved by kinase autophosphorylation) or *cis* (achieved by specific DNA strand orientation and sequence bias) [35]. Activation by these dual processes represents a potentially powerful mechanism by which DNA-PK protects DNA ends to maintain genome integrity. After the DSB repair, Ku likely remains trapped on the DNA [36]. How Ku gets removed from the DNA is not clear, although a protease-mediated degradation of Ku80 has been speculated [36].

Cellular and molecular targets of DNA-dependent protein kinase in non-homologous end joining

Many proteins have been listed as excellent *in vitro* and *in vivo* DNA-PK targets, but the functional relevance of their phosphorylation by DNA-PK remains mostly unclear. Most of the proteins involved in NHEJ (XRCC4, Ku70, Ku80, Artemis, DNA-PK_{cs}, and XLF) are excellent *in vitro* and *in vivo* targets of DNA-PK [2,37-40]. When a DSB occurs, the Ku heterodimer (Ku80/Ku70) first binds to the broken ends by using Ku80 and then recruits the DNA-PK_{cs}, which is activated upon binding to Artemis nuclease, and the repair process is completed by XRCC4-DNA ligase IV [39,41] (Figure 1). Physical association of DNA-PK_{cs} and its enzymatic activity are required for Artemis's endonucleolytic activity [39]. In the absence of DNA damage, Artemis is complexed with DNA-PK_{cs}. It has been shown that DNA-PK_{cs} is targeted to Ku-bound DNA; Artemis is released from DNA-PK_{cs} and is rebound again only when the kinase is activated [34]. Artemis itself has both endo- and exonuclease activities [39].

DNA-PK_{cs} strongly suppresses the exonuclease activity of Artemis but allows limited endonucleolytic trimming, likely at regions of transition from single-strand to double-strand [39,42].

Non-homologous end joining and DNA-dependent protein kinase in neurons

Mature neurons are essentially post-mitotic and do not proliferate, whereas some glial cells can undergo replication especially as a response to stress or damage [43,44]. Neurons are also among the most metabolically and transcriptionally active cells (reviewed in [45]), thus making these cells vulnerable to risks that involve DNA damage.

DNA repair pathways in brain have been studied extensively over the last two decades (reviewed in [45,46]). In mammals, DSB repair uses two mechanisms: HR and NHEJ. NHEJ is the predominant dsDNA repair pathway in mammalian cells [47]. Compared with the HR, NHEJ is considered error-prone and imprecise as it acts at the DNA break sites to restore the chromosomal structural integrity which could come at the expense of one or a few nucleotides. Since most of the higher eukaryote genome is non-coding, error-prone rejoining of DSBs by NHEJ generally has minimal deleterious consequences. However, DSB repair in coding regions can potentially introduce functionally important coding changes. Over time, as in aging, these small errors can accumulate, resulting in genome instability that leads to cellular dysfunction or death. Accordingly, it has been reported that 10% of p53 mutations in human cancers could be attributed to deletions arising from NHEJ sites [48]. NHEJ is also the predominant form of dsDNA repair pathway in post-mitotic neurons [49] and is critical in the nervous system development since mice deficient in DNA ligase IV, XRCC4, Ku70, and Ku 80, which are participants in the NHEJ event, show massive apoptosis of post-mitotic neurons [46,50]. Loss of NHEJ activity in the developing brain can be prenatally lethal and, in adults, can lead to neurodegenerative diseases [46,51,52]. Mice with defective NHEJ show accelerated aging [53,54].

DNA-dependent protein kinase and cell-cycle re-entry in neurodegeneration

One of the factors contributing to neurodegeneration is the re-entry of terminally differentiated post-mitotic neurons into the cell cycle because of chronic or acute insults associated with DNA damage and oxidative stress that result in apoptosis [55,56]. DSB repair capability is critical for neurogenesis during development, and damaged neurons demonstrate this by escaping apoptosis, re-entering the cell cycle, and incorporating into the developing brain, leading to neurodegeneration in mice with low or no ATM activity [57]. While ATM

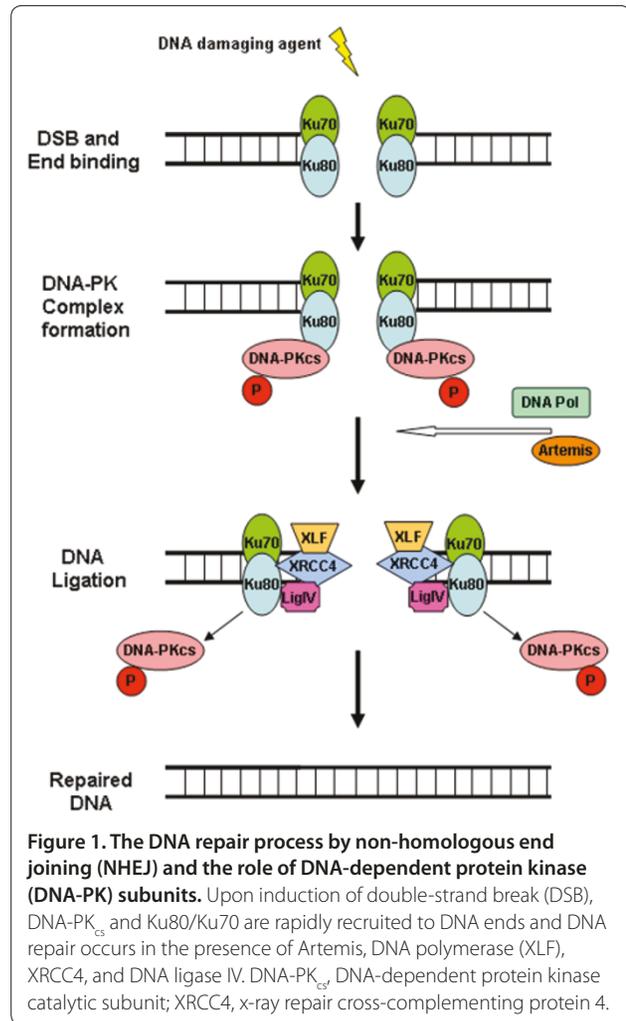
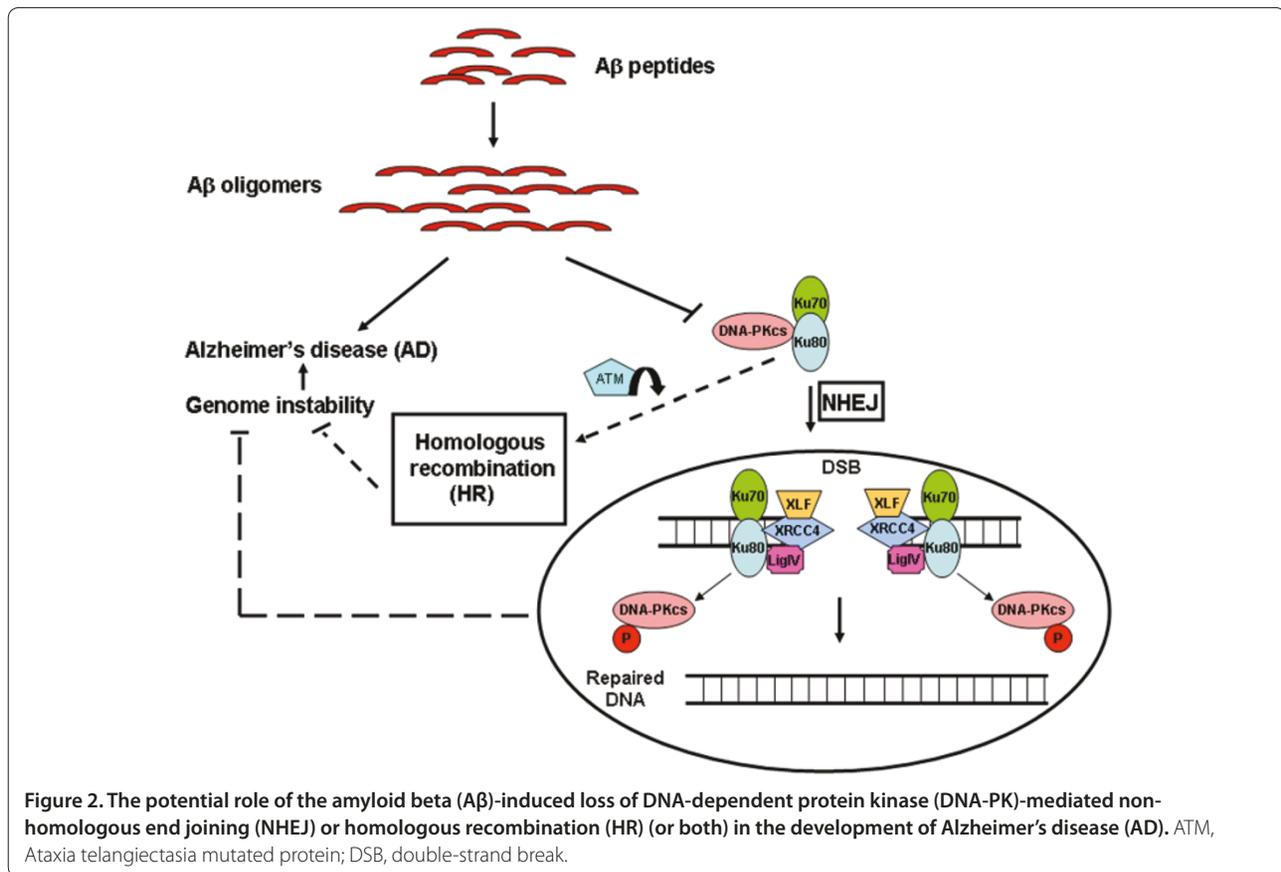


Figure 1. The DNA repair process by non-homologous end joining (NHEJ) and the role of DNA-dependent protein kinase (DNA-PK) subunits. Upon induction of double-strand break (DSB), DNA-PK_{cs} and Ku80/Ku70 are rapidly recruited to DNA ends and DNA repair occurs in the presence of Artemis, DNA polymerase (XLF), XRCC4, and DNA ligase IV. DNA-PK_{cs}, DNA-dependent protein kinase catalytic subunit; XRCC4, x-ray repair cross-complementing protein 4.

deficiency suppresses the re-entry of post-mitotic neurons into S-phase and protects against apoptosis [56], it also increases the yield of unrepaired DNA that eventually may be lethal. Neuronal DNA damage is linked to the re-entry of neurons into the cell cycle [56,58]. When post-mitotic neurons try to re-enter the cell cycle, the very attempt to transcribe a subset of cell cycle-related genes that have not been transcribed for years in the lifetime of a mature neuron may accumulate damaged DNA, which could trigger neuronal apoptosis [59].

Furthermore, it has been suggested that DNA replication resulted when cell-cycle re-entry preceded neurodegeneration in AD brains [60]. Reactive oxygen/nitrogen species are reported to cause unscheduled and incomplete DNA replication known as 'replication stress' [61]. Thus, inefficient DNA replication posing 'replication stress' in AD pathogenesis leading to genomic instability potentially links A β accumulation and erroneous cell-cycle pathways [62]. Obviously, incomplete DNA replication due to DSBs or defective DNA repair systems (or



both) would be highly probable in post-mitotic neurons, causing replication stress and subsequently leading to accelerated accumulation of further DNA damages and genomic instabilities [63,64]. Stalled replication forks collapse to yield one-ended DSBs, or 'double-strand ends', and abnormal DNA replication in post-mitotic neurons may be the source of intracellular increase in DNA content observed in AD brains [60,65]. It has been shown that DNA-PK_{cs} mutant cells fail to arrest replication following stress [66]. Additionally, studies show that, in response to replication stress-induced DNA damage, DNA-PK phosphorylates replication protein A (RPA) and dissociates RPA:DNA-PK complex [67,68], thereby inhibiting HR [69]. Thus, reduced DNA-PK activity in a cell could potentially induce replication stress and genome instability.

DNA-dependent protein kinase in Alzheimer's disease and aging

It has also been shown that cells from old mice contain more DSBs than cells from young mice and that the fidelity and efficiency decline significantly during cellular senescence [70]. This event may contribute to age-related genomic instability and aging. DNA-PK plays critical roles in, first, detecting DNA damage and, then,

triggering signaling pathways, including programmed cell death [53]. Ku80^{-/-} mice are defective in the NHEJ and telomere maintenance and show premature aging, but surprisingly no human disorder caused by Ku80 deficiency or mutation has been reported [54,71]. Interestingly, Ku80 and DNA-PK_{cs} protein levels as well as the DNA-binding ability of Ku80 are reduced following severe ischemic injury, which causes extensive neuronal death in rabbits [72]. Furthermore, though not significantly different from that of the age-matched controls, Ku-DNA binding is reduced in extracts of post-mortem AD mid-frontal cortex, and this could be attributed to reduced levels of Ku subunits and DNA-PK_{cs} [73]. However, a report from the same laboratory demonstrated that NHEJ is reduced in cortical extracts from brains of AD versus normal subjects and that DNA-PK_{cs} level was significantly lower in the AD brain extracts [74]. Whether other DNA repair systems, especially HR, are altered in the AD brains is not known (Figure 2).

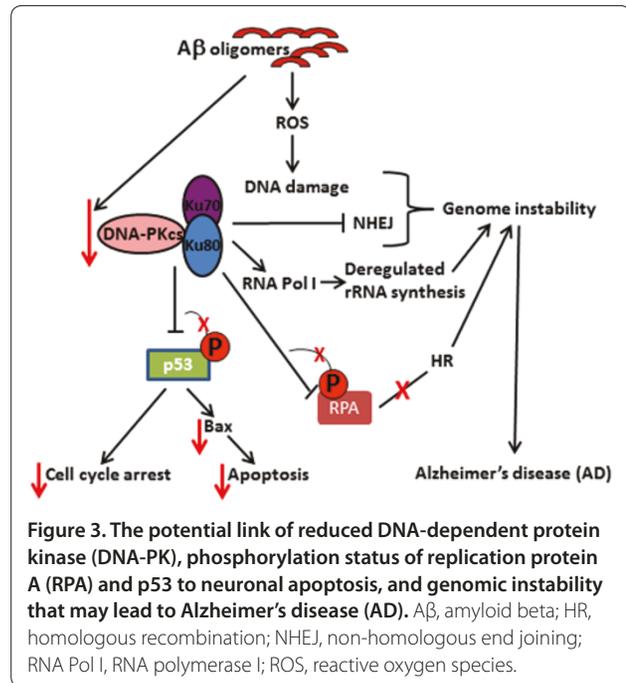
To explain the complexity of AD, a 'two-hit hypothesis' for AD development has been reported; the first hit makes neurons vulnerable and the second hit triggers the neurodegenerative process [75]. The first hit may constitute abnormalities when neurons try to re-enter the cell cycle or oxidative stress, which, if persistent, can create a

pro-oxidant environment as encountered in pre-AD and AD cases. In this environment, proteins highly sensitive to redox modulation, including p53, can be compromised [76]. A number of post-mortem studies suggest an involvement of p53 in AD, and high levels of p53 in certain neurons in post-mortem samples from patients with AD have been reported (reviewed in [77]). DNA-PK activates p53 by phosphorylating the amino-terminal site [78], and p53 can induce Bax, a pro-apoptotic protein that translocates to the mitochondria and initiates the intrinsic death pathway [79]. Regulation of Bax-mediated neuronal death also reportedly involves Ku70 phosphorylation by DNA-PK [80]. In this regard, reduction in DNA-PK_{cs} levels in AD brains does not seem to be consistent with the role of DNA-PK_{cs} as the trigger for p53-mediated neurodegeneration (Figure 3).

DNA-PK is believed to have little or no effect on p53-dependent cell-cycle arrest. In contrast, there are reports linking p53 phosphorylation by DNA-PK to cellular death machinery (reviewed in [81]). DNA-PK is also involved in regulating the activities of RNA polymerase I and II via phosphorylation (reviewed in [81]). Given these important substrates of DNA-PK that are critical players in cell death and gene transcription, it is difficult to pinpoint the exact role(s) of DNA-PK_{cs} and its cofactor (Ku80/Ku70) in AD. Likewise, it would be simplistic to directly link reduced levels of DNA-PK subunits and consequently less proficient NHEJ in AD brains to neurodegeneration. On the other hand, it is attractive to speculate that DNA damage (for example, induced by reactive oxygen species (ROS) downstream of A β) in neurons with reduced NHEJ activity, triggering them to re-enter the cell cycle unsuccessfully, could lead to the accumulation of excessive genomic damage and eventually cause neuron death (Figure 3). In either pathway, given that NHEJ is the process involved, the importance of the DNA-PK_{cs}/Ku complex in the development of neurodegenerative pathology may be considerable. The reduced levels of DNA-PK_{cs} and Ku80/Ku70 subunits in post-mortem AD brains may be perceived as upstream events of neuron loss in AD, although further studies to differentiate between cause and consequence are warranted.

DNA-dependent protein kinase and amyloid beta

In a recent study, sublethal levels of aggregated A β (25-35) have been shown to inhibit DNA-PK activity in nerve growth factor (NGF)-differentiated PC12 cells [82]. In this study, one of the potential mechanisms appears to be A β -induced ROS-mediated degradation of DNA-PK_{cs}. A β also induces DNA-PK_{cs} carbonylation, an irreversible oxidative protein modification that may trigger its degradation by proteasomes [83,84]. DNA-PK activity is also inhibited by H₂O₂ in cell-free assays, indicating that



ROS may directly inhibit DNA-PK activity [82]. On the other hand, A β (1-42), which can enter the nucleus of PC12 cells, also downregulates DNA-PK activity, possibly by a mechanism other than the involvement of oxidative stress. In AD cases, a decrease in DNA-PK_{cs} expression in neurons and astrocytes, though not significant, has been reported [85]. Although it is tempting to link AD development to A β -induced attenuation of DNA-PK activity and hence to reduced NHEJ activity, it may be argued that this event is a consequence rather than the prime cause, a simultaneous event that could occur independently of A β -triggered neurotoxic pathways.

Conclusions

In contrast to other NHEJ and HR factors, all three components of the DNA-PK complex (DNA-PK_{cs}, Ku80, and Ku70) are exceptionally abundant proteins, especially in human cells [86]. Given the complexity of AD, a clear distinction is lacking as to whether the expression of DNA-PK subunits may have been transcriptionally impaired in AD brains because of a hitherto unknown upstream event that is too generic to have a specifically targeted effect on DNA-PK. As for the reduced level of DNA-PK_{cs}, A β -induced proteasome-mediated degradation of DNA-PK_{cs} has been proposed [83,84]. With regard to other NHEJ components as essential as DNA-PK (for example, Artemis), their status in AD remains unexplored. In AD, it is possible that with already-declining NHEJ activity due to the defects in some other components associated with the process, a reduced DNA-PK activity may be consequential or a secondary

effect. Therefore, a decline in DNA-PK subunit levels and its kinase activity may, for the time being, serve as biomarkers until future studies, especially *in vivo* studies, add to substantiate a direct link of DNA-PK to AD. Furthermore, increased HR of substrates in cells that lack DNA-PK [87,88], co-localization of NHEJ and HR factors at the same DNA lesion [89], partial rescue of the severe phenotypes associated with deficiency of XRCC4 or ligase IV, by deletion of DNA-PK [90,91], indicate that, in the absence of DNA-PK and NHEJ, the cells may tend to acquire an alternate path (for example, HR) to repairing DSBs. Interestingly, in cell culture studies, crosstalk between HR repair and NHEJ has been shown to involve ATM, DNA-PK, and ATR, indicating that DNA-PK may participate in HR by co-regulating p53 and RPA [92]. Should such alternate pathway(s) be adversely affected by factors causing AD onset, the neurons devoid of any ability to repair DSBs may be vulnerable to degeneration. Since DNA-PK_{cs} and Ku mutations in humans are not existent [54,71] and aging brains also exhibit reduced levels of Ku80 and NHEJ activity [73,74], it remains to be seen whether reduced levels of DNA-PK complex and the enzyme activity do bear any direct relationship to AD (Figure 2). Discerning between normal aging-related attenuation of DNA-PK activity/expression and that in AD cases warrants careful assessment.

Lately, linking genomic lesions during neurodegeneration to transcriptional insufficiency is fast emerging [93]. Nucleolar insensitivity to DSBs has been implicated in neurodegeneration [93]. Nucleolus is the site of ribosomal RNA (rRNA) biogenesis [94]. The RNA polymerase I (Pol I) drives the transcription of rRNA, and continuous Pol I activity is required for nucleolar maintenance. The DNA-PK component Ku has been shown to suppress RNA Pol I transcription *in vitro* and in P19 stem cells [95-97]. In this context, a reduction in DNA-PK activity and level of expression of its components in AD brains [73,74] should act as a relief factor for Pol I transcription. Interestingly, while hippocampal neurons have been shown to have AD-associated reduction in nucleolar volume [98], in subjects with moderate AD pathology without cognitive impairment, nucleolar hypertrophy has been reported in both cortical and hippocampal neurons [98]. Although the latter scenario appears consistent with reduced DNA-PK and Ku levels in AD brains [73,74], the link remains unclear and needs further research.

Abbreviations

Aβ, amyloid beta; AD, Alzheimer's disease; ATM, Ataxia telangiectasia mutated protein; ATR, Ataxia telangiectasia and Rad3-related protein; BER, base excision repair; DNA-PK, DNA-dependent protein kinase; DNA-PK_{cs}, DNA-dependent protein kinase catalytic subunit; DSB, double-strand break; dsDNA, double-strand DNA; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; Pol I, RNA polymerase I; ROS, reactive oxygen species; rRNA, ribosomal RNA; RPA, replication protein A; XLF, x-ray repair cross-complementing protein 4-like factor; XRCC4, x-ray repair cross-complementing protein 4.

Competing interests

The author declares that she has no competing interests.

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