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DNA-PK and P38 MAPK: A Kinase Collusion in Alzheimer's Disease?

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Abstract

The pathogenesis of Alzheimer's disease (AD), characterized by prevalent neuronal death and extracellular deposit of amyloid plaques, is poorly understood. DNA lesions downstream of reduced DNA repair ability have been reported in AD brains. Neurons predominantly use a mechanism to repair double-strand DNA breaks (DSB), which is non-homologous end joining (NHEJ). NHEJ requires DNA-dependent protein kinase (DNA-PK) activity. DNA-PK is a holoenzyme comprising the p460 kD catalytic subunit (DNA-PK$_{cs}$) and its activator Ku, a heterodimer of p86 and p70 subunits. Ku first binds and then recruits DNA-PK$_{cs}$ to double-stranded DNA ends before NHEJ process begins. Studies have shown reduced NHEJ activity as well as DNA-PK$_{cs}$ and Ku protein levels in AD brains suggesting possible contribution of unrepaired DSB to AD development. However, normal aging brains also show reduced DNA-PK$_{cs}$ and Ku levels thus challenging the notion of any direct link between NHEJ and AD. Another kinase, p38 MAPK is induced by various DNA damaging agents and DSB itself. Increased DNA damage with aging could induce p38 MAPK and its induction may be sustained when DNA repair is compromised in the brain with reduced DNA-PK activity. Combined, these two events may potentially set the stage for an awry nervous system approaching AD.

Keywords: Ku; DNA repair; NHEJ; ROS; Amyloid beta

Abbreviations: AD: Alzheimer's Disease; ATM: Ataxia Telangiectasia Mutated Protein; ATR: Ataxia Telangiectasia and Rad3-related Protein; BER: Base Excision Repair; Cdk5: Cyclin Dependent Kinase 5; DNA-PK: DNA-Dependent Protein Kinase; DSB: Double Strand Breaks; DSBR: Double Strand Break Repair; HR: Homologous Recombination; MAPK: Mitogen Activated Protein Kinase; ERK: Extracellular Signal-Regulated Kinase; MKK6: Mitogen-activated Protein Kinase Kinase 6; NER: Nucleotide Excision Repair; Lig IV: Ligase IV; NHEJ: Non Homologous End Joining; NFT: Neurofibrillary Tangle; rRNA: Ribosomal RNA; SSBR: Single Strand Break Repair; XLF: XRCC4 Like Factor; XRCC4: X-ray Repair Cross Complementing Protein 4.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease affecting ~24 million people worldwide and this number could double by 2030 [1]. AD is characterized by specific neuronal death with accumulated neurofibrillary tangles (NFT) and extracellular amyloid beta (Aβ) deposits [2]. These characteristics are accompanied by memory impairment, cognitive decline and synaptic dysfunction [3]. Aβ which is produced by serial cleavage of the amyloid precursor protein (APP), directly injures neurons of the neocortical and limbic system [4]. By indirectly activating the microglia that release pro-inflammatory cytokines and reactive oxygen species (ROS), Aβ exerts additional neurotoxic effects [5,6]. Other contributors to AD pathogenesis include apolipoprotein E genotype [7]; neurofilament and Tau hyperphosphorylation [8-11], and Aβ generation [12]. AD being a multifactorial disease [13], no single factor has been identified as the main contributor to its development [14-17]. In various studies, oxidative stress has been linked to AD pathogenesis [18-20]. Oxidative stress can cause DNA damage, alter the levels and activity of DNA-repair proteins [21,13]. Thus, oxidative stress can induce cellular damage by ROS generation, and elevated levels of oxidative damage in DNA are observed in AD brains [22]. Cellular DNA damage and repair follow a homeostatic process, but as the cells age, the damage exceeds repair consequently disrupting the homeostasis [23,24]. Aging, a major risk factor in AD, is associated with cumulative oxidative stress and it is proposed that elevated levels of oxidized nucleic acids in neurons can lead to neuronal dysfunction in AD patients [25-27] thus

linking oxidative damage to neurodegeneration [28]. Accumulation of DNA damage due to dysfunctional DNA repair machinery can create events contributing to AD [29-32]. Furthermore, studies show that some human hereditary genetic defects in the DNA repair system are apparent during early onset and progressive neurodegeneration [33,34]. Understanding the factors responsible for DNA repair defects can unravel potential intervention points of AD pathogenesis caused by genome instability.

DNA repair, DNA-PK and Aβ

DNA damage induces the expression and activity of many kinases including the members of the PI-3 kinase family [35]. One of these kinases, the DNA-dependent protein kinase (DNA-PK) preferentially phosphorylates the serines (S) and threonines (T) of its target substrates [36]. DNA-PK is a holoenzyme comprising a catalytic subunit (DNA-PK$_{cs}$) of p460 and a regulatory subunit (Ku) of 70 kD (Ku70) and 80 kD (Ku80) heterodimer. Ku possesses the ability to bind to DNA ends [37,38]. DNA-PK plays a role in transcription, DNA recombination, and DNA repair [39-42]. When not associated with DNA-PK$_{cs}$, Ku independently binds DNA ends in a sequence-independent manner [43]. However, Ku is essential for targeting DNA-PK$_{cs}$ to the damaged DNA sites in living cells [44]. Studies show that double strand DNA breaks (DSB) can activate DNA-PK activity both in trans (*via* kinase autophosphorylation) or cis (*via* specific DNA strand orientation and base sequence) modes [45-47].

DNA repair pathways used by cells include base excision repair (BER), nucleotide excision repair (NER), single strand break repair (SSBR), and double strand break repair (DSBR). Double strand

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break (DSB) being the most lethal, in eukaryotes, two major DSB repair pathways operate; non-homologous end joining (NHEJ) and homologous recombination (HR). In higher order organisms, NHEJ functions as the predominant pathway for DSBR throughout the cell cycle [48-50], whereas HR functions are limited to the S and G2 stages of the cell cycle [51]. Specifically, DNA-PK actively engages in accessing the DNA ends during NHEJ [52,53].

NHEJ is the predominant dsDNA repair pathway in mammalian cells [54] and is more error-prone compared to HR as it acts at the DNA break points and the ensuing repair process can result in a loss of one or a few nucleotides. Fortunately, most of the higher eukaryote genome is non-coding. Therefore, errors resulting from DSB repair by NHEJ rarely lead to detriments. Nonetheless, with aging, these non-detrimental errors eventually can cause genome instability upon progressive accumulation, and cause cell death or dysfunction. It is important to note that 10% of p53 mutations in human cancers reportedly occurred due to deletions resulting from compromised NHEJ [55]. Mature post-mitotic neurons do not undergo proliferation [56,57], but they are one of the most metabolically and transcriptionally active cells [58]. Due to this reason, these neurons can be more vulnerable to DNA damage-induced injury. In post-mitotic neurons, since NHEJ is the predominant pathway for DSB repair [59,60], mice deficient in DSB repair pathway components (DNA Ligase IV, XRCC4, Ku70 and Ku 80) (Figure 1) show robust apoptosis of these neurons [12,61]. Furthermore, mice with defective NHEJ undergo accelerated aging. Severity of the loss of NHEJ activity in the developing brain manifests in prenatal lethality and adult neurodegenerative diseases [12,62,63].

Post-mitotic neurons that are terminally differentiated, when triggered to re-enter cell cycle following chronic or acute insults inducing DNA damage and/or oxidative stress, undergo apoptosis [64,65]. Neurons re-entering cell cycle are prone to accrue DNA damage [65,66]. Therefore, it is possible that DNA replication is a consequence of cell cycle re-entry that precedes neurodegeneration in AD brains [67]. In addition, reactive oxygen/nitrogen species can cause misdirected and inefficient DNA replication, called 'replication stress' [68,69], which during AD pathogenesis can lead to genomic instability thus facilitating Aβ accumulation and deregulation of cell cycles. In post-mitotic neurons, these adverse events can be further amplified with the existence of defective DNA repair systems leading to accumulation of additional DNA damages and genomic instabilities [70,71]. It is plausible that intracellular increase in DNA content reported in AD brains [72,67] could originate from these dual events. In fact, it has been reported that DNA-PK$_{cs}$ mutant cells under stress undergo non-arrested replication [73]. Also suggested is a possibility that accumulated single-stranded DNA (ssDNA) at replication forks may create aberrant DNA structures leading to DSBs that activate DNA-PK [74]. Based on these findings, it is apparent that neurons deficient in DNA-PK activity could sustain unhindered replication stress leading to genome instability (Figure 1). In physiologic conditions, one of the most important roles DNA-PK plays is sensing the DNA damage [75] and then, inducing signaling pathways that can lead to programmed cell death [76]. In Ku80$^{-/-}$ mice, defective NHEJ and telomere maintenance with premature aging have been reported [77,78]. Reductions in Ku80 and DNA-PK$_{cs}$ protein levels as well as Ku80's DNA-binding ability following severe ischemic injury leading to neuronal death in rabbits have also been shown [79]. Furthermore, although not significantly different from the age-matched controls, reduced Ku-DNA binding in extracts of post-mortem AD mid-frontal cortex suggests a potential link to reduced levels of Ku and DNA-PK$_{cs}$ proteins [80]. Reduced DNA-PK$_{cs}$ expression in neurons and astrocytes of AD brains as well as

in age-matched controls has been reported [81]. Compared to normal subjects, reduced NHEJ activity in the cortical extracts of AD brains and significantly lower levels of DNA-PK$_{cs}$ in the AD brain extracts have also been reported [82] suggesting a critical role of DNA-PK-mediated NHEJ in brain health.

Other than its essential role in NHEJ, since DNA-PK is also a critical player in cell survival/death and gene transcription, it is compelling to directly link reduced levels of DNA-PK and Ku with less proficient NHEJ to neurodegeneration occurring in AD brains. Already challenged with this condition, additional DNA damage caused by agents such as ROS (Figure 1) to the neurons may misdirect them to re-enter cell cycle albeit unsuccessfully, which in turn can lead to accumulation of excessive genomic damage causing neuronal death. Therefore, it's most likely that reduced levels of DNA-PK$_{cs}$ and Ku80/Ku70 create the detrimental upstream event before the advent of AD [83].

The mechanism of how reduced DNA-PK activity may be linked to Aβ is best shown in *in vitro* studies. For example, in NGF-differentiated PC12 cells, sub-lethal levels of aggregated Aβ 25-35 inhibited DNA-PK activity as did hydrogen peroxide [84]. It is likely that Aβ -induced ROS-mediated DNA-PK$_{cs}$ degradation *via* carbonylation, an irreversible oxidative protein modification [85,86]. Conversely, cultured hippocampal neurons from severe combined immunodeficient (SCID) mice that lack DNA-PK activity have been shown to be hypersensitive to apoptosis upon exposure to Aβ [87]. In a normal individual however, whether Aβ induced attenuation of DNA-PK activity compromising NHEJ is linked to the onset of AD awaits examination.

P38 MAPK and Aβ

The mitogen-activated protein kinase (MAPK) family of serine/threonine kinases are activated by extracellular stimuli, such as growth factors, cytokines, hormones and cellular stresses and thus regulate a number of major cellular processes including cell growth, differentiation and survival [88]. One of the major MAPK family members is, p38
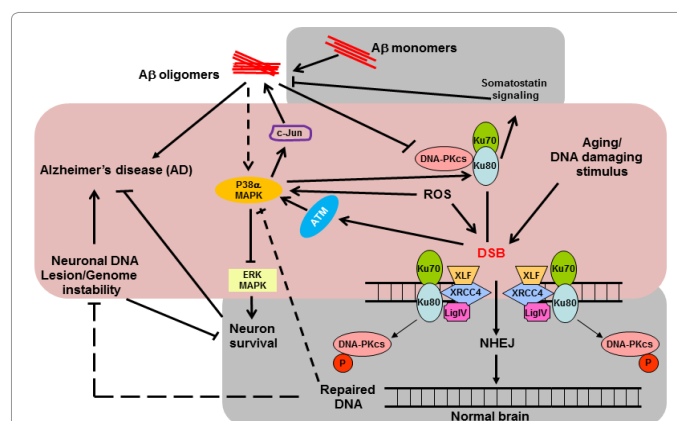

Figure 1: Schematic presentation of a potential link of DNA double strand breaks (DSB), DNA-PK and p38α MAPK in normal and AD brains. Upon induction of DSBs either by normal aging/ROS or other DNA damaging agents, Ku80/Ku70 andDNA-PK$_{cs}$ and are rapidly recruited to DNA ends, and DNA repair occurs as it would in normal brains. However, in AD brains, in addition to formation of Aβ oligomers from Aβ peptides, sustained DSBs in the genome would cause genome instability leading to the loss of normal neuronal activity. Additionally, with depleted DNA-PK activity andNHEJ, sustained DSBs could activate p38α MAPK *via* ATM triggering neuronal death, potentially mediated by one of the downstream pathways being ERK MAPK down regulation and another *via* c-jun activation. Disruption of somatostatin signaling *via* Ku80 (a somatostatin receptor) depletion may also lead to Aβ oligomerization, a prime trigger of AD. Shaded areas show normal (gray) and deregulated sequences of events (purple).

MAPK [89]. P38 MAPK activation can have both beneficial and adverse effects on cell growth and survival depending on the cell type and p38 MAPK subtype; for example, activated p38 MAPK pathway has an anti-apoptotic effect during neuronal differentiation, but is pro-apoptotic in mature neurons exposed to various stresses such as excitotoxic stimuli [90,91]. Various DNA damaging agents including UV irradiation and ionizing radiation can induce p38 MAPK activity [92,93]. Activation of p38 MAPK by DSB-inducing agents occurs *via* phosphorylation of p38 MAPK itself [94].

Significantly higher level of activated p38 MAPK along with the activation of its upstream activator, mitogen-activated protein kinase kinase 6 (MKK6), have been reported in early stages of AD [95,96]. In the amyloid precursor protein (APP)-transgenic mice Aβ accumulation with increased p38 MAPK activity has been reported [97-99]. Aβ induces p38 MAPK activity (Figure 1) through activation of pro-inflammatory cytokines [100-102]. Inhibition of p38 MAPK activity suppressed expression of pro-inflammatory cytokines and consequently attenuated synaptic dysfunction causing behavioral alterations in an AD mouse model [103]. Knocking down p38α MAPK in neurons from APP/presenilin 1(PS1) double transgenic mice that overexpress human mutated APP and PS1 enhanced autophagy and promoted BACE1 (β-site APP-cleaving enzyme) degradation resulting in reduced Aβ generation [104].

Activated-p38 MAPK has been shown to co-localize with cyclin-dependent kinase 5 (Cdk5) in Tg2576 mice, a murine model of AD [105]. Cdk5 inhibition has been shown to be more neuroprotective compared to p38 MAPK/c-jun inhibition, which suggests that Cdk5 may act upstream regulating neurodegenerative pathways triggered by p38 MAPK [90]. Deregulated Cdk5 triggers ROS generation [91], and ROS is known to activate p38 MAPK (Figure 1) [90,106,107]. Activated p38 MAPK upregulates its direct downstream target, c-Jun, a pro-apoptotic transcription factor that can potentially cause neuro-apoptosis in AD [90]. Studies have shown that c-Jun expression is up-regulated in AD [108]. Furthermore, c-Jun not only induces abnormal Aβ generation in AD *via* activation of the β-APP gene [29] but also promotes apoptosis in hippocampal neurons treated with Aβ [109,110]. Not surprisingly, neurons of c-Jun-null mice are resistant to Aβ-mediated toxicity [111]. Thus, activation of p38 MAPK, either by DSB or Aβ or both (Figure1), can potentially injure the neurons by inducing further Aβ generation and activating its target c-jun, a pro-apoptotic transcription factor.

Activated p38 MAPK has been reported during early onset of AD [112]. Up-regulation of p38 MAPK also occurs during microglial inflammation [113]. Activated p38 MAPK localized to the NFT and the dystrophic neurites in AD brain [114]. However, a direct link between p38 MAPK activation and NFT formation was ruled out indicating that p38 MAPK activation could cause neurodegeneration independent of NFT formation [112]. Moreover, since activated p38 MAPK was present at a higher level in some early AD cases, but was noticed at a modest level in a few severe AD cases, it has been suggested that early activation of p38 MAPK may be critical at early stages of AD [112]. *In vitro* studies have suggested that p38 MAPK dysregulation causes tau hyper-phosphorylation and formation of NFT in AD [115,116]. There is evidence that AD patients might benefit from p38 MAPK inhibitors [117]. Although p38α and p38β inhibition has been shown to improve Aβ-induced long-term potentiation deficits [117,118], it also increases hyperexcitability in the APP transgenic mice [118]. Therefore, the specific effects of p38 inhibitors remain unclear [119,120]. Furthermore, roles of other p38 kinases, p38γ and p38δ, in AD are not known. Recently, a beneficial role of p38γ in AD was reported in mice showing T205 phosphorylation of tau by p38γ to be partly responsible for inhibiting Aβ toxicity [121]. In this context, determining threshold levels of specific activated p38 MAPK that can switch from a beneficial role in neuronal differentiation and development [122] to trigger adverse effects on the neuron may hold the key to understanding its contribution to AD.

Conclusion

Alteration in brain pathology in AD occurs many years prior to the manifestation of clinical cognitive decline [123]. AD is a multifactorial disease and several factors contribute to its development. Identifying these factors or multiple pathways that go awry poses formidable challenges, as do attempts to link the specificity of these abnormalities to functional outcomes in the patients. For example, compared to other players associated with NHEJ, all three components of the DNA-PK complex (DNA-PK$_{cs}$, Ku80 and Ku70) are abundantly expressed in human cells [124] and how a reduction in any of the DNA-PK components in the brain that is also observed even in normally aging individuals [82,80] compromises NHEJ so as to contribute to AD development is intriguing. Given the complexity of AD, it is imperative to take into consideration additional factors such as p38 MAPK activation that could accentuate the effects of DNA-PK deficiency in an otherwise normally aging individual. Ku80 is also a specific somatostatin receptor [125] and can regulate DNA-PK activity through somatostatin signaling pathway [81]. It has been speculated that Ku80 deficiency, therefore, can disrupt somatostatin signaling leading to Aβ generation (Figure 1) [42]. During normal aging, since DNA-PK components are reduced, unrepaired DSBs could occur albeit at a level not sufficient to induce sustained p38 MAPK activation. In contrast, in AD cases, a threshold level of DSBs could induce p38 MAPK activation subsequently amplifying Aβ generation. Understanding the subtlety of the occurrences of these events would justify a deficiency of NHEJ during aging to be normal whereas NHEJ deficiency along with p38 MAPK activation to be pathogenic. Early prediction of AD might depend on capturing the timing of the onset of multiple pathway defects, such as DNA-PK, p38 MAPK and somatostatin signaling. This would not only help discern between normal aging-related events and triggers of AD but also enable identifying potential intervention points.

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