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Hunster Yang

Western University, hyang322@uwo.ca

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Western University

Abstract

The prevalence of neurodegenerative diseases, such as Alzheimer's disease (AD), has been progressively increasing each year, in which AD is now the sixth leading cause of death in the United States (Alzheimer's Association, 2017). The effects of neurodegenerative diseases present a massive challenge for society and health care systems globally, and as a result, examining the pathogenesis of these diseases have been a common aim throughout past literature. Nonetheless, the molecular mechanisms that underlie neurodegenerative diseases are still fundamentally undetermined, yet studying to understand the pathogeneses can provide extensive information for the development of curative treatments. In particular, fully understanding the protein eukaryotic initiation factor (eIF2) can provide insight into the molecular pathways that may give rise to neurodegenerative disorders. Through the critical review of two research articles, this paper investigates the association between eIF2 and neurodegenerative diseases, specifically AD and prion disease, in efforts to understand the molecular mechanisms that underlie these disorders. Through critically analyzing Ma et al. (2013) and Moreno et al. (2012), it is evident that the eIF2 pathway plays a vital role in the pathogenesis of these neurodegenerative disorders. Incorporating the findings from both studies together, it can be proposed that with further research, the proteins within the eIF2 process can possibly be used as targets for novel therapies to treat these disorders. These two articles provide vast future directions for further research to extend upon, in order to reveal more regarding the significance of eIF2 signalling in mitigating synaptic failure, neuronal loss, behavioural dysfunctions, and ultimately battle the massive global challenge that is presented by neurodegenerative diseases.

A Critical Review on the Association between eIF2 and Neurodegenerative Diseases

The prevalence of neurodegenerative diseases, such as Alzheimer's disease (AD), has been progressively increasing each year, in which AD is now the sixth leading cause of death in the United States (Alzheimer's Association, 2017). The effects of neurodegenerative diseases present a massive challenge for society and health care systems globally, and as a result, examining the pathogenesis of these diseases have been a common aim throughout past literature. Nonetheless, the molecular mechanisms that underlie neurodegenerative diseases are still fundamentally undetermined, yet studying to understand the pathogenesises can provide extensive information for the development of curative treatments. Neurodegenerative diseases such as AD, Parkinson's disease, Huntington's disease, and prion diseases have different clinical, pathological and biochemical conditions, however they all share notable similarities that involve the accumulation of misfolded proteins, as well as mechanisms that result in neuronal loss (Halliday & Mallucci, 2013). In particular, fully understanding the unfolded protein response (UPR) can provide insight into the molecular pathways that may give rise to these disorders. Specifically, eukaryotic initiation factor (eIF2) has been widely emphasized in previous research to having a vital role in this process, along with its connection to neurodegenerative diseases. Through the critical review of two research articles, this paper aims to investigate the association between eIF2 and neurodegenerative diseases, specifically AD and prion disease, in efforts to understand the molecular mechanisms that underlie these disorders.

Neurodegenerative diseases are characterized by the gradual death of nerve cell populations, along with the accumulation of protein aggregates (Barnham, Masters, & Bush, 2004). In particular, AD is the most common cause of dementia, which is characterized by issues with memory, thinking and behaviour (Holtzman, Morris, Goate, 2011). Throughout past

literature, there have been numerous approaches to determining the molecular pathways involved in AD. For instance, previous research has focused on the accumulation of amyloid plaques in the brain, which are derived from amyloid- β ($A\beta$) peptides, and how these plaques are related to the development of AD (Golde, Eckman, & Younkin, 2000; Hardy & Selkoe, 2002). Likewise, Holtzman and colleagues (2011) found that the pathogenesis of AD also includes the development of neurofibrillary tangles (NFTs) of the protein Tau, along with the loss of synapses and neuronal cell death. Cell death can be attributed to prolonged activation of the UPR due to increased endoplasmic reticulum stress (Lindholm, Wootz, & Korhonen, 2006). Therefore, examining essential proteins involved in the UPR pathway such as eIF2, along with their contribution to synaptic dysfunction and memory impairments, can provide greater knowledge of the development of AD.

In similar ways, prion disease, another neurodegenerative disorder, has been heavily explored in the disciplines of neuropathology and biochemistry. Prion diseases are caused by the buildup of the misfolded forms of the prion protein (PrP), which leads to neurodegeneration and neurological defects (Eikelenboom et al., 2002). The pathogenesis of prion diseases involves the conversion of normal, cellular prion protein (PrP^C) to abnormal, misfolded, protease-resistant prion protein (PrP^{Sc}) that accumulate into aggregates in the brain (Prusiner, 1991). As a result, prion diseases cause increased levels of PrP^{Sc} , resulting in prolonged activation of the UPR, which consequently reduces the levels of synaptic proteins (Lindholm et al., 2006; Prusiner, 1991). The decline of synaptic proteins could be due to increased protein degradation or decreased protein synthesis. However, prion infections have been discovered to hinder the ubiquitin proteasome system, thus reducing protein degradation, suggesting that the reduction of protein levels are from decreased protein synthesis (Kristiansen et al., 2007). Therefore,

investigating proteins such as eIF2, which are involved in molecular mechanisms that lead to decreased protein synthesis, can offer greater understanding of prion diseases, in order to ultimately develop treatments targeting prion infections.

The protein eIF2 is essential in regulating protein synthesis through its contribution in the UPR (Cao & Kaufman, 2012). To be specific, whether or not the α subunit of eIF2 is phosphorylated by kinases or dephosphorylated by phosphatases have distinct and extensive effects on translational control in cells. Phosphorylated eIF2 α (eIF2 α -P) causes downstream cell signalling pathways that generally inhibit protein synthesis. Since neurodegenerative diseases, such as AD and prion disease, are characterized by neuronal cell death which is related to prolonged activation of UPR, exploring the relationship between eIF2 α -P and the development of these neurodegenerative diseases is vital. The following two critical reviews attempt to demonstrate that eIF2 α -P certainly plays a vital role in the pathogenesis of AD and prion disease. In turn, these two articles provide a basis where future investigations can extend upon, to further explore the association between eIF2 α -P and neurodegenerative diseases, in order to ultimately discover potential therapeutic targets for treatments.

In a study conducted by Ma and colleagues (2013), their main objective was to explore whether or not inhibiting eIF2 α kinases could improve synaptic plasticity and spatial memory in AD model mice. Specifically, they hypothesized that through genetically removing eIF2 α kinases PERK and GCN2, levels of eIF2 α -P will reduce, and as a result prevent AD-associated deficits in synaptic plasticity and memory. In their investigation, there were three primary goals addressed: PERK removal prevented A β -mediated defects, PERK deletion relieved abnormalities in AD mice, and the elimination of GCN2 alleviated AD-associated deficits.

Ma et al. (2013) first wanted to verify the differing levels of eIF2 α -P in AD and wildtype (WT) mice, as well as examine the A β -linked effects of removing PERK. In order to investigate the role of eIF2 α , they analyzed eIF2 α -P levels in various brain areas of AD model mice. AD mice were transgenic, characterized by the overexpression of amyloid precursor protein (APP). Through western blots targeting eIF2 α -P, they found increased eIF2 α -P levels in the hippocampus and prefrontal cortex (PFC), but not in the cerebellum. Therefore, they confirmed that AD mice contained abnormally high concentrations of eIF2 α -P. In addition, they explored whether A β -linked impairments in synaptic plasticity and memory could be improved by deleting PERK. Using WT mice that had a floxed PERK gene and Cre recombinase, they were able to knockout PERK in excitatory hippocampal neurons. They found that in WT mice, LTP-inducing high-frequency stimulation (HFS) resulted in dephosphorylation of eIF2 α , and thus increases in protein synthesis. However, in the presence of A β , LTP was inhibited in WT mice. In mice that had PERK removed, A β had no effect on LTP or hippocampal synaptic plasticity since it demonstrated similar LTP activity as WT mice. Therefore, by removing PERK, they found that A β -induced deficits in hippocampal synaptic plasticity were reduced.

Ma and associates (2013) then explored the effects of decreasing eIF2 α -P levels by genetically removing kinases (e.g., PERK) on protein synthesis, memory and synaptic plasticity. This time, they created AD transgenic mice that had floxed PERK genes and Cre recombinase, to knockout PERK. They analyzed the effects of PERK deletion on protein synthesis in hippocampal slices. In AD mice, they found reduced translation mediated by eIF2 α -P, whereas in AD mice with PERK deleted, the reduction of protein synthesis was prevented. Hence, they discovered that PERK deletion stabilizes the effects on protein synthesis mediated by eIF2 α phosphorylation. Furthermore, the researchers investigated how the removal of PERK would

impact spatial learning and memory. They tested the mice on three separate behavioural tasks: Morris water maze, object location, and Y water maze. AD mice demonstrated compromised learning and memory abilities by exhibiting decreased escape latency, greater target quadrant occupation, along with increased platform crossings. Conversely, AD mice that had PERK deleted did not display these spatial memory impairments. Additionally, they explored whether removing PERK could improve the synaptic plasticity defects. They measured synaptic plasticity through examining LTP; in AD mice, LTP was significantly reduced, while the deletion of PERK resulted in normal LTP. Therefore, removing PERK in AD mice restored LTP and alleviated synaptic plasticity deficits.

Moreover, Ma and associates' (2013) last primary goal was to study the effects on memory and synaptic plasticity through the deletion of GCN2, another eIF2 α kinase. In similar ways, they found analogous results to the findings from deleting PERK, since the removal of GCN2 also reduced LTP failure and spatial memory deficits. Integrating all the findings from this article, it is clear that eIF2 α -P plays a vital role in AD, since the suppression of eIF2 α kinases resulted in counteractive AD-associated effects on protein synthesis, memory, and synaptic plasticity. Investigating the molecular mechanisms that are responsible for AD-related synaptic failure and memory impairments could offer innovative targets for AD treatments; for instance, future research on potential therapies that target specifically PERK and GCN2 have potential in relieving AD symptoms.

In another study, Moreno and colleagues (2012) explored the association between eIF2 α -P and neurodegeneration from prion disease. The general aim of the investigation was to demonstrate that translational repression of global protein synthesis was mediated by eIF2 α -P, and plays a role in prion neurodegeneration. They expected that synaptic failure and neuronal

loss found in prion-infected mice were attributed to the constant inhibition of translation that was mediated by increased levels of eIF2 α -P. Therefore, they also predicted that promoting translation by reducing eIF2 α -P levels could be neuroprotective and reduce neuronal death. In this study, they attempted to address six primary goals. In order to test the relationship between eIF2 α -P and prion disease, they used transgenic mice that expressed PrP levels three times greater than WT levels, and infected them with prion disease.

Moreno et al. (2012) first determined the molecular mechanisms involved with the development of prion disease. Through numerous western blots, they found that throughout the progression of the disease, PrP^{Sc} levels increased gradually, while there were significant reductions in synapse proteins, synapse numbers, synaptic transmission, and burrowing behaviour at 9 weeks post infection (w.p.i.) in prion-infected mice. Consequently, they then explored the cause of this reduction in protein synthesis observed at 9 w.p.i., and discovered that levels of PERK-P and eIF2 α -P increased at 9 w.p.i. Since PERK-P phosphorylates eIF2 α and eIF2 α -P prevents the initiation of translation, they concluded that eIF2 α -P mediates the significant reduction of protein synthesis found at 9 w.p.i.

In addition, analyzing polysomal fractions and northern blots, Moreno et al. (2012) investigated the precise effects of increasing eIF2 α -P levels at 9 w.p.i. They found that when eIF2 α -P levels grew, abrupt decreases in protein synthesis were observed through substantial reduction in global translation rates. Moreover, they then used different transgenic mice that expressed varying levels of PrP (i.e., 1x WT, 3x WT, 6x WT) and analyzed the relationship between eIF2 α -P and the onset of neurodegeneration. They discovered parallel findings in all three strains of mice, where PrP^{Sc} levels increased, then a rise in levels of eIF2 α -P were detected, followed by reductions in synapse numbers and synaptic protein levels.

Furthermore, Moreno and associates (2012) explored the effects of whether reducing eIF2 α -P levels could lead to neuroprotective effects. They used two lentiviral-mediated methods that targeted the hippocampus of prion-infected mice. They aimed to overexpress GADD34, an eIF2 α -P phosphatase that reduced eIF2 α -P levels directly, as well as perform targeted RNA interference of PrP to abolish UPR activation and prevent phosphorylation of eIF2 α . Both the overexpression of GADD34 and PrP knockdown caused reductions in eIF2 α -P levels, preventing the translational suppression, thereby restoring global translation rates in prion-infected mice. Additionally, synaptic protein and transmission levels were similar to WT levels, along with increased burrowing behaviour and an enhanced survival rate. Therefore, they discovered that decreasing eIF2 α -P levels resulted in neuroprotective properties. In opposition, they examined whether increased levels of eIF2 α -P would exacerbate prion neurotoxicity through injections of salubrinal, an inhibitor of eIF2 α -P dephosphorylation. They found expected results, where salubrinal increased eIF2 α -P levels, reduced global translation rates, decreased synaptic protein levels, and ultimately accelerated death rate.

There are wide implications that can be drawn from all the findings presented in the study by Moreno et al. (2012). Linking all the results together, it is evident that eIF2 α -P causes repression of translation, and in turn, is associated with the onset of prion neurodegeneration. This article definitely provides a basis for future research to further investigate the mechanisms underlying prion disease, to ultimately explore novel therapies that target possibly eIF2 α -P, in order to treat neurodegenerative disorders that involve misfolded proteins.

To critically evaluate the article by Ma and colleagues (2013), there are numerous aspects that strengthened their study, in which their findings altogether support the conclusions as well as address the original goal. Nonetheless, there are several limitations, though importantly, this

study provides future directions for research to improve the weaknesses of this investigation. One strength of their study is that their results clearly demonstrate that the removal of PERK or GCN2 indeed affects pathways in AD mice, and in turn influences protein synthesis, synaptic plasticity and memory. Their experiments followed a coherent order; for example, they confirmed that deleting PERK decreased PERK expression and eIF2 α -P levels, and as a result, the reduction of protein synthesis mediated by eIF2 α -P was prevented. One thing to appreciate is that they definitely provided substantial evidence for preventing AD-related effects by removing an eIF2 α kinase. However, it cannot be concluded with certainty that the deletion of an eIF2 α kinase was the sole factor that caused AD-associated synaptic plasticity and memory impairments to be alleviated. For instance, they focused on the effects of removing one specific eIF2 α kinase PERK, and found improvements to spatial memory performance in AD mice. In contrast, a study by Zhu, Henninger, McGrath and Cavener (2016) found that PERK may indeed have a role in memory formation. Therefore, these two competing schools of thought suggest that PERK/eIF2 α -P signalling is actually very complex, and is possibly involved in many mechanisms that all work together, to ultimately give rise to diverse cellular activities.

Another limitation of the study by Ma et al. (2013) is that they only explored the effects in the hippocampus, however AD has been shown to affect numerous other regions, such as amygdala, cerebellum, and PFC (Holtzman et al., 2011). Ma and associates (2013) also confirmed increased levels of eIF2 α -P within the PFC, in addition to the hippocampus of AD mice. Thus, a crucial question of whether removing eIF2 α kinases would have similar effects in the PFC was not addressed. Similarly, they only investigated A β -induced deficits, yet AD is also characterized by NFTs of the Tau protein (Holtzman et al., 2011). Interestingly, Ohno (2014) found that the PERK pathway contributes to the hyper-phosphorylation of Tau, producing NFTs.

Therefore, this introduces a possibility in which removing PERK not only improves A β -induced deficits, but may also prevent NFT-related impairments. This brings into question the actual molecular mechanism that governs the alleviation of AD-related defects from deleting PERK. Lastly, they did not analyze the effects of eIF2 α phosphatases, as they may also provide relief to AD-related deficits. Nonetheless, the study by Ma and colleagues (2013) overall has vital implications scientifically and clinically, in addition to serving as a groundwork experiment for future research to build upon.

The experiment conducted by Moreno and associates (2012) also includes numerous strengths, in which their findings provide extensive support for how eIF2 α -P mediates translational inhibition, and how it is linked to prion neurodegeneration. It is evident that the findings address the goals of the study because through manipulating the levels of eIF2 α -P in prion-infected mice, signs of neurodegeneration were seen. The procedure that the researchers used to explore translational control by eIF2 α -P and neuronal death can be appreciated, since they first confirmed that at 9 w.p.i., significant reductions in synapse numbers and protein levels were found. After, they determined that interestingly at 9 w.p.i., eIF2 α -P levels increased, which caused the reduction in synaptic protein levels since eIF2 α -P regulates inhibition of protein synthesis. They then focused and manipulated eIF2 α -P levels at 9 w.p.i. to observe its association with neurodegeneration. Thus, this study followed a logical process that effective in analyzing the role of eIF2 α -P. However, a limitation is that they solely focused on 9 w.p.i., yet at 7 w.p.i. was when they first observed significant reductions in synapse numbers. Hence, the paper failed to address the question of what happened in between 6 w.p.i. to 9 w.p.i., that may have caused reductions in synapse numbers at 7 and 9 w.p.i. The study intended to analyze the molecular mechanisms which underlie prion neurodegeneration, but falls a little short as it left

several essential questions unanswered, specifically in regards to the progression leading up to synaptic changes in prion-infected mice. Furthermore, another critique of the paper is that the experiment did not take into account other factors that may influence the eIF2 α pathway. As suggested in previous studies, eIF2 α signalling is extremely intricate, and can have numerous influences that affect this pathway (Wek, Jiang, & Anthony, 2006; Zhu et al., 2016). Future studies should explore the effects of changing eIF2 α -P levels by altering the expression of eIF2 α kinases or phosphatases, and how that may impact prion disease. Despite the limitations to the investigation conducted by Moreno et al. (2012), the findings clearly demonstrate the role of eIF2 α in repressing translation, and how this mechanism is linked with prion neurodegeneration.

In conclusion, the critical analyses of the investigations by Ma et al. (2013) and Moreno et al. (2012) provide extensive evidence for greater understanding of the mechanisms that are responsible for AD and prion diseases. It is evident that the eIF2 α pathway plays a vital role in the pathogenesis of these neurodegenerative disorders. Incorporating the findings from both studies together, it can be proposed that with further research, the proteins within the eIF2 α process can possibly be used as targets for novel therapies to treat these disorders. These two investigations tested mice models, however after additional research, treatments that target this mechanism have potential to be implicated in clinical settings. Additionally, examining the link between other neurodegenerative disorders and the eIF2 α pathway could be valuable, to ultimately discover more effective therapies. Nonetheless, these two papers provide vast future directions for further research to extend upon, in order to reveal more regarding the significance of eIF2 α signalling in mitigating synaptic failure, neuronal loss, behavioural dysfunctions, and ultimately battle the massive global challenge that is presented by neurodegenerative diseases.

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