Nrf2 and Alzheimer's Disease

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Contributor: Don Davies

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor that reduces oxidative stress. When reactive oxygen species (ROS) or reactive nitrogen species (RNS) are detected, Nrf2 translocates from the cytoplasm into the nucleus and binds to the antioxidant response element (ARE), which regulates the expression of antioxidant and anti-inflammatory genes. Nrf2 impairments are observed in the majority of neurodegenerative disorders, including Alzheimer's disease (AD). The classic hallmarks of AD include β-amyloid (Aβ) plaques, and neurofibrillary tangles (NFTs). Oxidative stress is observed early in AD and is a novel therapeutic target for the treatment of AD. The nuclear translocation of Nrf2 is impaired in AD compared to controls. Increased oxidative stress is associated with impaired memory and synaptic plasticity. The administration of Nrf2 activators reverses memory and synaptic plasticity impairments in rodent models of AD. Therefore, Nrf2 activators are a potential novel therapeutic for neurodegenerative disorders including AD.

NF-κB neurodegeneration oxidative stress reactive oxygen species inflammation

1. Introduction

Oxidative stress is involved with the occurrence and progression of AD. Aβ elevation is associated with increased levels of oxidation products from proteins, lipids and nucleic acids in the hippocampus and cortex of humans with AD [1]. In contrast, lower Aβ levels in the brain are correlated with lower oxidative stress markers [2]. Aβ plaques can reduce Ca 2+ storage in the endoplasmic reticulum, which results in an excess of Ca 2+ in the cytosol [3]. Due to the excess of cytosolic Ca 2+ , glutathione (GSH) levels are decreased and reactive oxygen species (ROS) can accumulate in the neurons [4]. The oxidative stress in AD patients may be a result of excitotoxicity from the glutamatergic N -methyl- d -aspartate (NMDA) receptors. NMDA receptor activation in AD has been shown to result in an excessive influx of Ca 2+ by increasing cell permeability and generation of ROS and reactive nitrogen species [5][6]. In addition, Aβ can initiate free radical formation by activating NADPH oxidase [7]. Furthermore, abnormal aggregates composed of p-Tau protein lead to increased ROS production in AD. ROS was the key result of impaired axonal transport and caused by abnormal p-Tau protein [8].

Nrf2 is a key endogenous modulator in the protection against oxidative stress. In response to oxidative stress, Nrf2 translocates from the cytoplasm into the nucleus and activates genetic expression with antioxidant activity. AD patients had less nuclear Nrf2 in the CA1 region of their hippocampus than the controls despite oxidative stress markers in the hippocampal neurons of patients with AD [9]. This indicates that Nrf2 was not translocating from the cytoplasm into the nucleus in hippocampal neurons in patients with AD, despite oxidative stress markers in these neurons and an abundance of nuclear Nrf2 in the neurologically normal age matched controls (see **Figure 1**).

Therefore, some process may be blocking Nrf2 nuclear activity, which may contribute to neuronal dysfunction. The levels of cytoplasmic Nrf2 are not different between age-matched controls and patients with AD. Albeit, the nuclear impairment is not the result of a general loss of Nrf2 protein but could reflect dysfunctional nuclear trafficking. Since the two hallmarks of AD are misfolded proteins, Aβ plaques and NFT, it is likely that endoplasmic reticulum stress is active in the hippocampus during the progression of AD, which may alter the Nrf2 pathway in the hippocampus. Methylene blue treatment in a mouse model of tauopathy increased the activation of Nrf2 and reduced tauopathy and oxidative stress [10]. Treatment with methylene blue was also associated with improved behavior with reduced locomotor abnormality, reduced anxiety abnormality, and improvement in learning and memory. Therefore, methylene blue may be a novel treatment option for people with AD because methylene blue reduces tau, which is one of the hallmarks of AD.

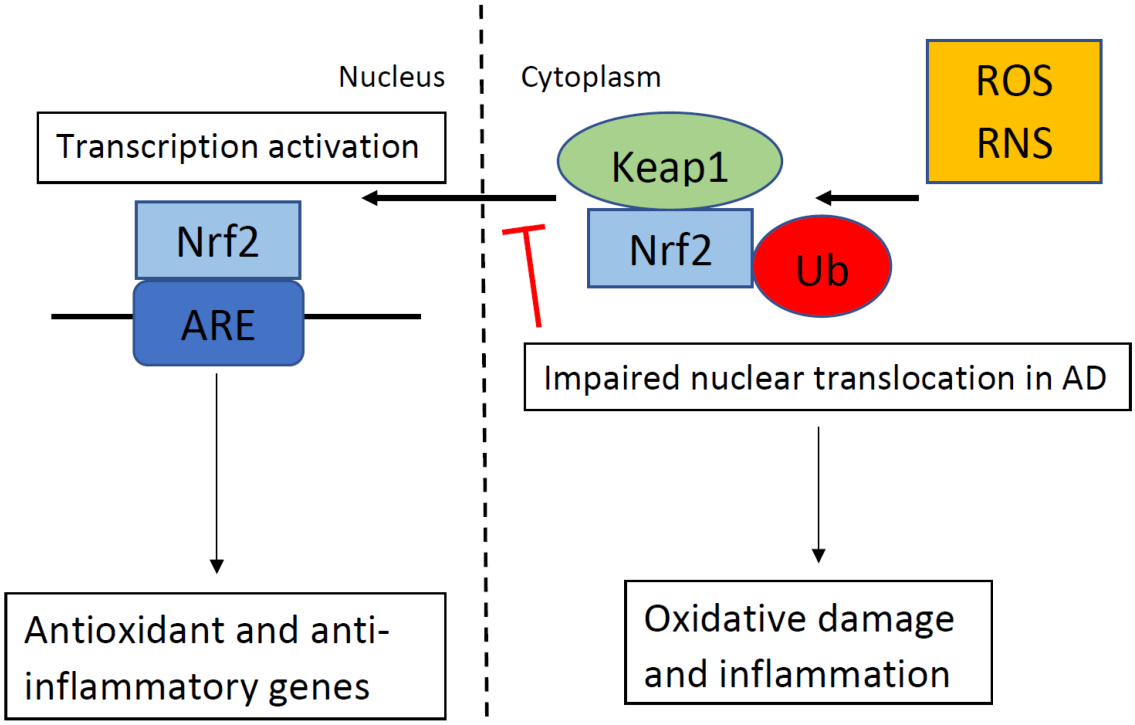

Figure 1. In the absence of oxidative stress. Kelch-like ECH-associating protein 1 (Keap1) suppresses the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and targets Nrf2 for ubiquitination (Ub). When Keap1 detects oxidative stress via reactive oxygen species (ROS) or reactive nitrogen species (RNS), as shown in the top right portion of the figure, Keap1 ends the inhibition of Nrf2, and Nrf2 translocates into the nucleus. Nrf2 binds to the antioxidant response element (ARE), which regulates the expression of antioxidant and anti-inflammatory genes. In Alzheimer's disease (AD), nuclear translocation in response to ROS/RNS is impaired, which results in oxidative damage and inflammation.

Initially, ROS was thought to only have negative physiological effects. However, others have observed the beneficial effects of ROS on mitochondria and in various cellular pathways [11][12]. Low levels of ROS are shown to have beneficial effects while high levels of ROS are associated with AD, suggesting a threshold determines whether ROS is beneficial or harmful [13]. The low levels of ROS regulate various cellular pathways, such as H2O2 regulating various signaling pathways with proteins containing cysteine residues [14]. Given the beneficial effects of

low levels of ROS, Nrf2 activators should only be considered when ROS levels have crossed the threshold from beneficial into harmful.

2. Cross-Talk between the NF-κB and Nrf2 Signaling Pathways

Nrf2 signaling contributes to the anti-inflammatory process by regulating target genes via the antioxidant response element (ARE) and Keap1 system [15]. The Keap1/Nrf2/ARE signaling pathway mostly regulates the expression of anti-inflammatory genes and ultimately blocks the progression of inflammation [15]. Oxidative-stress mediated NF-κB activation can be blocked by the Keap1/Nrf2/ARE pathway [16][17]. NF-κB impacts the Keapl/Nrf2/ARE signaling pathway in three parts. First, Keap1 degrades IKK, which prevents the phosphorylation of NF-κB [16]. Second, oxidative stress, which activates IKK leads to phosphorylation of NF-κB and translocation of NF-κB from the cytoplasm into the nucleus stimulates the production of proinflammatory cytokines such as IL-1, IL-6, TNF-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) [18][19]. Ultimately, COX-2 reacts with Keap1 and activates Nrf2, which leads to the suppression of oxidative stress-mediated NF-κB activation [20]. Third, Nrf2 binds to CBP and other transcriptional machinery to begin ARE-driven gene transcription [21]. However, NF-κB inhibits Nrf2 activation by competing with Nrf2 for CBP and ultimately reducing ARE gene expression [21]. Overall, the Keap1/Nrf2/ARE signaling pathway inhibits the production of proinflammation [20]. Moreover, it has been demonstrated that Nrf2 directly regulates the expression of anti-inflammatory mediators such as CD36, IL-17D, macrophage receptor, and G protein-coupled receptor (GPCR) kinase, which suppresses the progression of inflammatory responses [22][23][24]. Nrf2 induces the anti-inflammatory phenotype of microglia and macrophages, while it decreases LPS-induced transcription of other NF-κB target genes [25][26]. Nrf2 increases cysteine and GSH levels in macrophages. However, depletion of GSH triggers macrophages to Nrf2 activation by LPS [27]. These findings show that Nrf2 acts as an anti-inflammatory marker, which is critical for regulating inflammatory responses.

Nrf2 and NF-κB Crosstalk with Other Transcription Factors

In addition to the cross talk between Nrf2 and NF-κB, there is cross talk among other immunomodulator transcription factors. Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor involved with the response to hypoxia and metabolism. HIF-1α is highly expressed in most innate and adaptive immune cells [28]. The HIF-1 and Nrf2 pathways are mediated by ROS and have many overlapping cellular pathways including vascular endothelial growth factor (VEGF), erythropoietin (EPO), and angiopoietin 2 (ANGPT2) [29]. The blockade of Nrf2 is associated with lowered HIF-1α at the post-translational level suggesting that Nrf2 is involved with the modulation of the prolyl hydroxylase domain containing proteins (PHDs) [30][31]. The Nrf2 genes are able to increase HIF-1 signaling, which may have resulted in poor colorectal cancer patient survival [32]. Hypoxic cellular conditions results in NF-κB activation in phagocytes, which activates HIF-1α [33][34][35]. HIF-1α increases neutrophil survival via activation of the NF-κB pathway, which results in persistent inflammation [36].

Activator protein 1 (AP-1) is a family of bZIP transcription factors consisting of two families of genes, Fos (c-Fos, FosB, Fra1, and Fra2) and Jun (c-Jun, JunB, and JunD) [37][38]. There is cross talk between AP-1 and Nrf2 with AP-

1 activation decreased from the Nrf2 activators SFN and EGCG [39]. NF-κB and AP-1 transcription factors are modulated via different mechanisms. However, they are both activated with many of the same stimuli [40]. Many genes are required for the coactivation of AP-1 and NF-κB, which suggests they are working together [41].

The signal transducer and activator of transcription 3 (STAT3) is a transcription factor involved with inflammation. Activation of Nrf2 increased the levels of small heterodimer proteins (SHP) resulting in STAT3 repression [42]. The STAT3-NF-κB complex in the fascin promoter contributes to transcription when exposed to IL-6 and TNF-α [43]. The nuclear factor of activated T cells (NFAT) is a family of transcription factors involved with immune response. The pathways between NFAT, Nrf2, and NF-κB interact on several regulatory steps and are involved with tumor development and chemoresistance in pancreatic cancer [44]. NF-κB and NFAT share similar DNA binding domains and fast nuclear translocation when activated [45]. FOXO are a group of the Forkhead family of transcription factors that have conserved DNA binding domains and have a key role in immunoregulation. The activation of FOXO via ROS results in gene expression for antioxidants and might attenuate the activity of Nrf2 [46]. The primary FOXO member, FOXO3a inhibits NF-κB activation via Th activation [47].

3. Conclusions

More research is required to investigate the potential linkage between two crucial transcription factors, Nrf2 and NF-κB in neurodegenerative diseases. It is believed that investigating this linkage will greatly assist in developing therapeutic choices for slowing and/or preventing the onset of neurodegenerative disorders such as AD; it will also assist in preventing memory and synaptic plasticity impairments.

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