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Interplay between Nrf2 and NF-κB in Neuroinflammatory Diseases

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Abstract

Many neurodegenerative conditions involve redox imbalance and neuroinflammation. Nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-κB (NF-κB) are key transcription factors regulating antioxidant and inflammatory pathways, respectively. These two opposing factors are inversely regulated, with activity of one most often accompanied by diminished activity of the other, giving rise to the oxidative stress and neuroinflammation evident in neurodegeneration. Emerging evidence is uncovering a complex interplay between Nrf2 and NF-κB, involving extensive interaction of regulatory mechanisms including cytosolic activators and repressors, transactivation partners and direct transcriptional crosstalk. Understanding these interactions may provide insight into the pathophysiology of neuroinflammatory diseases and facilitate discovery of novel therapeutic targets.

Abbreviations

AD: Alzheimer's Disease; ALS: Amyotrophic Lateral Sclerosis; AREs: Antioxidant Response Elements; Bcl-3: B-cell lymphoma 3 protein; CAM: Cell Adhesion Molecule; CBP: CREB Binding Protein; COX-2: Cyclooxygenase-2; FTH: Ferritin Heavy Chain; FTL: Ferritin Light Chain; GCLC: Glutamate-Cysteine Ligase Catalytic subunit; GCLM: Glutamate-Cysteine Ligase Modifier subunit; GSK3β: Glycogen Synthase Kinase 3 beta; GSTs: Glutathione-S-Transferases; HD: Huntington's Disease; HDAC3: Histone Deacetylase 3; HMOX1: Heme Oxygenase 1; IκB: Inhibitor of κB; IKK, IκB Kinase: IL-1: Interleukin-1; IL-2: Interleukin-2; IL-6: Interleukin-6; IL-8: Interleukin-8; iNOS: Inducible Nitric Oxide Synthase; Keap1: Kelch-like ECH-associated protein; LPS: Lipopolysaccharide; MCP1: Monocyte Chemotactic Protein 1; MS: Multiple Sclerosis; NF-κB: Nuclear Factor-κB; NQO1: NAD(P)H dehydrogenase (quinone) 1; Nrf2: Nuclear factor erythroid 2-related factor 2; PD: Parkinson's Disease; PDTC: Pyrrolidine Dithiocarbamate; RAC1: Rho family GTP-binding protein 1; TBI: Traumatic Brain Injury; TLR: Toll-like Receptor; TNFα: Tumor Necrosis Factor alpha; (β-TrCP): β-Transducin repeat-Containing Protein; VCAM: Vascular Cell Adhesion Molecule

Nrf2 Function and Activation

Nrf2 is the master regulator of antioxidant pathways, responsible for promoting the transcription of hundreds of antioxidant and cytoprotective genes, and in the brain appears to be repressed in neurons and predominantly restricted to glia [1,2]. Nrf2 is a cap'n'collar basic leucine zipper transcription factor. Under normal conditions, Nrf2 is bound in the cytosol by its negative regulator Kelch-like ECH-associated protein (Keap1), a substrate adaptor protein of a Cullin3-dependent ubiquitin E3 ligase complex. Keap1 constitutively targets Nrf2 for proteasomal degradation. When activated, Nrf2 dissociates from Keap1 and can translocate to the nucleus where it forms heterodimers with small Maf proteins and binds to antioxidant response elements (AREs), promoting the transcription of a battery of antioxidant and cytoprotective genes [3]. Nrf2 targets include genes for glutathione synthesis and utilization (e.g. GCLM, GCLC, GSTs), the thioredoxin and peroxiredoxin systems, NAPDH generation, iron metabolism (HMOX1, FTL, FTH) and quinone detoxification (NQO1) [4]. Nrf2 can be activated in response to oxidative stress and electrophiles via Keap1. Keap1 contains several highly sensitive cysteine residues that when oxidised cause Nrf2 to dissociate from Keap1, allowing Nrf2 to translocate to the nucleus. Thus Keap1 acts as a redox sensor [3]. Nrf2 activity is also regulated by kinases such as GSK3β [5].

Regulation of NF-κB Activation

NF-κB is a key regulator of the cellular inflammatory profile, promoting the transcription of inflammatory genes. In the brain, NF-κB signalling occurs predominantly in microglia, as well as astrocytes and oligodendrocytes, but not in neurons [6]. The NF-κB complex consists of homo- or heterodimers of p65, RelB, c-Rel, p50 and p52, the most abundant of which is p65/p50. The dimers are bound in the cytosol by a family of repressor proteins called inhibitor of κB (IκB), which include IκBα, IκBβ, IκBε, IκBγ and Bcl-3. IκB is phosphorylated by the IκB kinase (IKK) complex, consisting of IKKα, IKKβ and IKKγ subunits [7]. Upon activation by stimuli including TNFα, LPS and IL-1, IKK phosphorylates IκB, facilitating IκB degradation via the β-transducin repeat-containing protein (β-TrCP)-Skp1-Cullin1 ubiquitin ligase complex [8]. NF-κB homo- and heterodimers can then translocate to the nucleus and bind to κB motifs in the promoter region of proinflammatory target genes [9]. These include cytokines (e.g. IL-1, IL-2, IL-6, TNFα), chemokines (e.g. IL-8, MCP1), adhesion molecules (e.g. CAM, VCAM) and inducible effector enzymes (e.g. iNOS, COX-2) [7].

Inverse Regulation of Nrf2 and NF-κB in Neurodegeneration

Inflammation, with elevated pro-inflammatory mediators and oxidative stress, is common to many neurodegenerative diseases, as evidenced by the presence of activated astrocytes and microglia in the affected regions of the brain and/or spinal cord in diseases including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), generally associated with degenerating neurons. Activated microglia are also found within and around amyloid plaques in AD, while activated astrocytes surround the plaques. Multiple sclerosis (MS), particularly the relapsing-remitting form, presents with transient localized sites of inflammation, whereby infiltrating peripheral T cells and microglia attack axonal myelin sheaths and oligodendrocytes. Stroke and traumatic brain injury (TBI) involve glial and peripheral immune cell activation at the site of injury that varies as the injury progresses. This extensive neuroinflammation and oxidative stress is consistent with elevated NF-κB activity concomitant with impaired Nrf2 activity [10,11], hence there is abundant evidence for inverse regulation of Nrf2 and NF-κB in neurodegenerative disease. Indeed, Nrf2 expression is diminished in human post-mortem tissue of AD and ALS patients [12,13]. Further evidence for the interplay between Nrf2 and NF-κB comes from experimental induction of inflammation. LPS-induced inflammation in the hippocampus is exacerbated in Nrf2-deficient mice [14], while induction of inflammation via α-synuclein in BV2 microglia is enhanced in the absence of Nrf2 [15]. Deletion of Nrf2 enhances inflammation and is associated with worsened outcomes in animal models of AD [16,17], PD [18], MS [19], and TBI [20]. These studies show that the absence of Nrf2 enhances NF-κB activity.

Conversely, inducing Nrf2 is associated with decreased inflammation. Nrf2 inducers such as synthetic triterpenoids and dimethyl fumarate decrease neuroinflammation and are protective in animal models of AD [21], PD [22,23], ALS [24], MS [25,26], HD [27,28] and stroke [29]. Other Nrf2 inducers also reduce neuroinflammation and are protective in animal models of AD [30], PD [31], ALS [32] and TBI [33], as does overexpression of Nrf2 in AD [34], ALS [35] and PD [18] animal models. That induction of Nrf2 suppresses inflammation and conversely inhibition of Nrf2 exacerbates inflammation across these diverse diseases and models, each with inflammatory pathology differing in localisation, extent and temporal pattern, supports the idea that Nrf2 and NF-κB are inversely regulated in a fundamental manner.

In humans, dimethyl fumarate is approved for the treatment of relapsing-remitting MS under the trade name Tecfidera following two successful Phase III trials [36], while the Nrf2 inducer DL-3-n-butylphthalide has produced positive outcomes in phase II clinical trials for acute ischemic stroke (administered within 48h of stroke onset) and vascular cognitive impairment without dementia [37,38]. However, none of these trials specifically interrogated Nrf2 or NF-κB signalling. Interestingly, NF-κB is inhibited in B cells isolated from MS patients administered Tecfidera [39], and this inhibition seems to occur in cultured lymphocytes in a manner independent of Nrf2 [40]. Clearly Nrf2 and NF-κB activity are inversely related in neuroinflammatory diseases. The molecular mechanisms governing the interplay between Nrf2 and NF-κB will now be discussed (Figure 1).

Activation of Nrf2 and NF-κB Signalling by Oxidative Stress

Nrf2 is well known to be regulated by oxidative stress via oxidation of sensitive cysteine residues on Keap1, causing release of Nrf2 and subsequent nuclear translocation [3]. In addition to proinflammatory mediators, NF-κB is also believed to be regulated by oxidative stress. This stems from early investigations showing activation of NF-κB signalling by oxidants and inhibition of NF-κB signalling in the presence of antioxidants occurring at the level of NF-κB release from IκB [41-43]. This led to speculations that Nrf2 may impede NF-κB activity by eliminating oxidative stress. However, more subtle and specific interactions between oxidants/antioxidants and NF-κB are emerging [44]. For instance, inhibition of NF-κB signalling by antioxidants has been shown to occur independent of their antioxidant activity, such as N-acetylcysteine inhibiting TNFα stimulation of NF-κB via interference with the TNFα receptor. In the case of pyrrolidine dithiocarbamate (PDTC), a well-known inhibitor of NF-κB that prevents the dissociation of NF-κB from IκB [45], its inhibitory action was originally ascribed to its antioxidant actions [42]. More recently, the mechanism by which PDTC inhibits NF-κB has been shown to be via disruption of IκB ubiquitin ligase activity [46]. However, PDTC also activates Nrf2 in brain and cultured astrocytes but not neurons [2], and improves cognition in the APP/PS1 AD mouse model [47]. Whether Nrf2 activation by PDTC is driven by NF-κB inhibition, or by other independent mechanisms remains to be elucidated.

Promiscuity of Nrf2 and NF-κB Activators and Repressors

There is considerable interaction between Nrf2 and NF-κB activators and repressors. For instance, Nrf2 binds to Keap1 via a conserved ETGE motif in Nrf2. IKK also contains an ETGE motif and can bind to Keap1 [48-50]. Keap1-mediated degradation of IKK is demonstrated by the elevation of IKK and NF-κB signalling when Keap1 is knocked down [48,50]. Furthermore, while Keap1 knockdown is well-known to induce Nrf2 signalling (due to lack of repression) [3], the influence of Nrf2 *per se* can be excluded as co-knockdown of Nrf2 has no effect [48]. In contrast to the proteasomal degradation of Nrf2, Keap1-dependent degradation of IKK likely occurs via an autophagy-dependent pathway [50], as per the degradation of Keap1 itself [51]. Importantly, the ETGE motif in IKK is conserved across human, chimpanzee, chicken and dog, but not mouse, rat, xenopus or medaka fish [48,50], which may influence investigations of this relationship in many *in vitro* and preclinical animal models. The relative extent and conditions governing the binding of Keap1 to IKK and Nrf2 remains unclear.

Conversely, there is also crosstalk between the NF-κB-related E3 ligase adapter protein β-TrCP and Nrf2. Following phosphorylation by IKK, IκB releases NF-κB and is targeted for proteasomal degradation via the β-TrCP-Skp1-Cullin1 ubiquitin ligase complex in manner analogous to Nrf2 degradation via the Keap1-Cul3-Rbx1 complex [8]. Nrf2 can also be targeted for degradation in a Keap1-independent manner by β-TrCP, in a process mediated by GSK3β-induced phosphorylation of activated Nrf2, thereby shutting down Nrf2 activity [52,53]. As per IKK/Nrf2 interactions with Keap1, the relative competition of Nrf2 and IκB for β-TrCP requires further investigation.
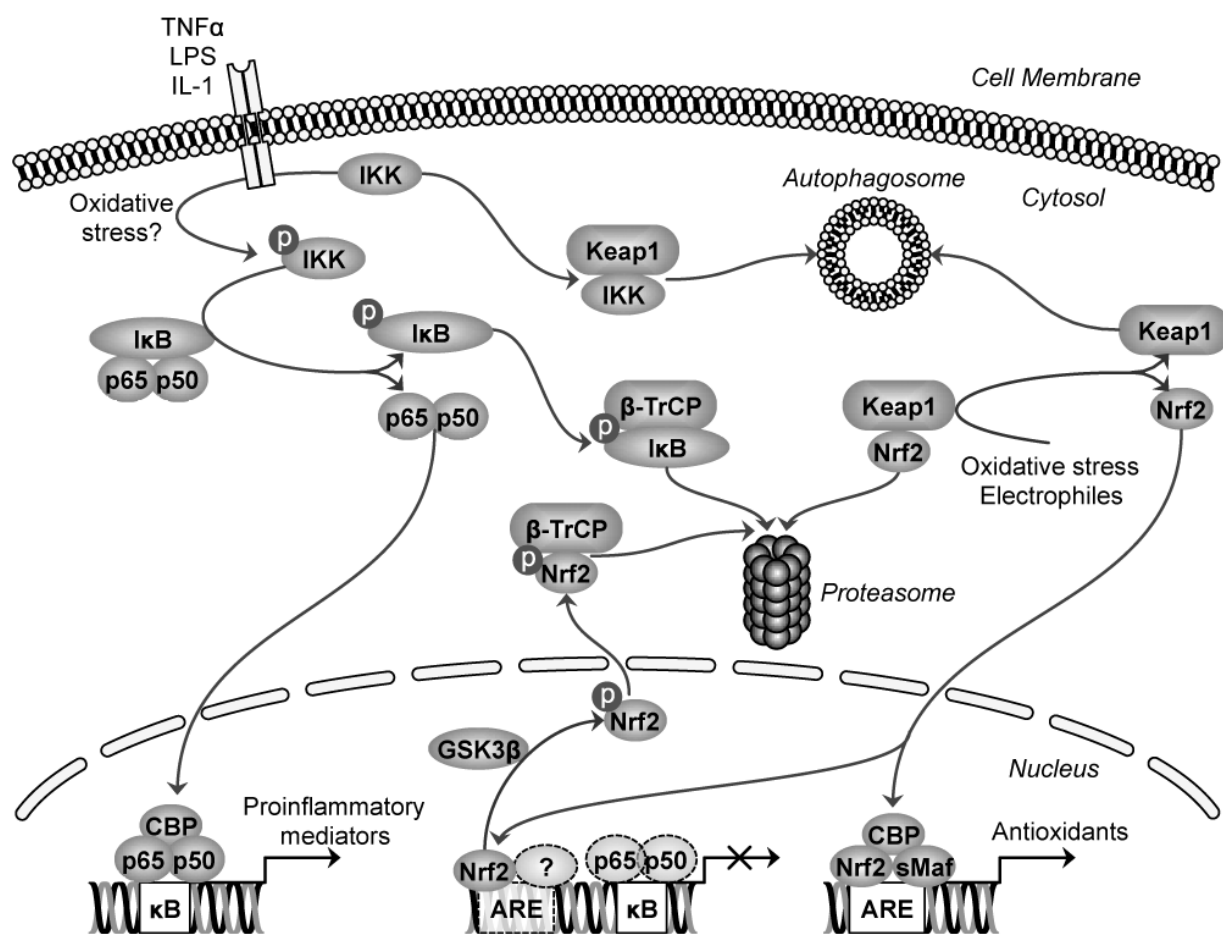
Figure 1: Interplay between Nrf2 and NF-κB regulation. NF-κB (depicted as p65/p50 heterodimer) is bound in the cytoplasm by IκB. In response to external stimuli or possibly oxidative stress, IKK is activated and phosphorylates IκB, causing dissociation of NF-κB which subsequently translocates to the nucleus where it promotes the transcription of proinflammatory genes by binding to κB motifs. Phosphorylated IκB binds to β-TrCP and is targeted for proteasomal degradation. Under normal conditions, Nrf2 is bound by Keap1 in the cytosol and also targeted for proteasomal degradation. Upon activation by oxidative stress or electrophiles, Nrf2 is release from Keap1 and can translocate to the nucleus where it forms a heterodimer with small Maf proteins (sMaf) and binds to antioxidant response elements (ARE) to promote the transcription of antioxidant genes. Nrf2 can also bind to NF-κB target genes and repress their transcription, in a process possibly involving binding partners and not influenced by presence or absence of the ARE sequence or NF-κB binding. Both NF-κB and Nrf2 compete for CBP, which promotes DNA binding. Activated Nrf2 can be phosphorylated by GSK3β, which facilitates binding by β-TrCP and targeting for proteasomal degradation. Keap1 is degraded by autophagy, and can also bind and degrade IKK via autophagy.

Activation of toll-like receptors (TLR), well known to induce an NF-κB-dependent inflammatory response, has recently been shown to activate Nrf2 via autophagy-dependent degradation of Keap1 [54]. Another NF-κB activating protein, Rho family GTP-binding protein (RAC1) [55,56], has recently been shown to also activate Nrf2, which subsequently blocks RAC1-dependent NF-κB activation [57]. Conversely, the Nrf2 target heme oxygenase-1 *per se* inhibits NF-κB activation in ischemic and haemorrhagic stroke [58,59].

Interactions between Nrf2 and NF-κB Transactivational Activity

There is also extensive crosstalk between Nrf2 and NF-κB in the nucleus. NF-κB can directly promote Nrf2 transcription via the κB motif in the promoter region of the NFE2L2 gene encoding Nrf2 [60].

Both transcription factors also compete with CREB binding protein (CBP), which assists in binding of the transcription factors to their cognate DNA motifs [61,62]. Phosphorylated p65 appears to have a higher affinity for CBP than Nrf2, outcompeting the latter and diminishing Nrf2 target transcription [62]. On the other hand, Nrf2 activation in microglial-like BV2 cells by ethyl pyruvate competes with p65 for CBP, suppressing p65 transcriptional activity [63]. Activated p65 can also repress Nrf2 signalling by promoting hypoacetylation of histones via HDAC3, which also sequesters CBP and/or the small Maf protein MafK, all repressing Nrf2 activity [62].

In addition to these many and varied mechanisms of co-regulation, direct transcriptional repression of NF-κB proinflammatory targets by Nrf2 has recently been described. Kobayashi et al. [64] find that Nrf2 binds to the promoter region of proinflammatory targets of NF-κB,

repressing their transcription. This appears to occur regardless of NF-κB binding, and does not require the ARE sequence, to which Nrf2 binding typically results in promotion of transcription [64]. Nrf2 binding to NF-κB targets may involve binding partners, although their identity remains to be determined. The mechanism by which Nrf2 represses transcription appears to involve inhibition of RNA Pol II recruitment, thus preventing transcription [64]. This landmark study identifies for the first time a mechanism by which Nrf2 transcriptional activity directly represses proinflammatory cytokine production. The study focuses on peripheral macrophages, and it remains to be determined whether this mechanism is maintained in inflammatory cells of the central nervous system.

Conclusions

Nrf2 and NF-κB control the transcription of redox balance and inflammatory pathways, respectively. Given the opposing nature of these pathways, it is not surprising that there is extensive crosstalk between almost every level of Nrf2 and NF-κB regulation. The idea that NF-κB is regulated by redox status, which in turn is controlled by Nrf2, is too simplistic and a more complex picture is emerging. It is likely that the balance of Nrf2 and NF-κB signalling is dynamically governed by a combination of external stimuli and the prevailing cellular and environmental conditions, specific to given cell types and tissues. The mechanisms modulating Nrf2 and NF-κB in neuroinflammatory diseases require further investigation, which could uncover new therapeutic targets for their treatment.

Acknowledgements

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