Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways

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Abstract

In most tissues, cells are exposed to frequent changes in levels of oxidative stress and inflammation. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor-κB (NF-κB) are the two key transcription factors that regulate cellular responses to oxidative stress and inflammation respectively. Pharmacological and genetic studies suggest that there is functional cross-talk between these two important pathways. The absence of Nrf2 can exacerbate NF-κB activity leading to increased cytokine production, whereas NF-κB can modulate Nrf2 transcription and activity, having both positive and negative effects on the target gene expression. This review focuses on the potentially complex molecular mechanisms that link the Nrf2 and NF-κB pathways and the importance of designing more effective therapeutic strategies to prevent or treat a broad range of neurological disorders.

The role of Nrf2 and its regulation

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a key transcription factor controlling many aspects of cell homoeostasis in response to oxidative and toxic insults. In particular, Nrf2 mediates basal and induced transcription of phase II antioxidant proteins, which are responsible for the clearance of reactive oxygen species (ROS), providing protection against the accumulation of toxic metabolites [1]. Among the most studied Nrf2-target genes are NAD(P)H dehydrogenase quinone 1 (NQO1), heme oxygenase-1 (HO-1), γ-glutamyl cysteine ligase modulatory subunit (GCLM), the catalytic subunit (GCLC) and ferritin, which function to maintain a reducing environment within the cell [2–4]. In total, Nrf2 drives transcription of hundreds of genes, which encode a multitude of proteins involved in diverse cellular functions, including protein and organelle homoeostasis [5,6]. Moreover, Nrf2 also plays a prominent role in orchestrating glucose metabolism by mediating transcription of components of the pentose phosphate pathway [7]. Cellular levels of Nrf2 are strictly regulated by multiple mechanisms, not all of which are fully defined. The best characterized mechanism of Nrf2 regulation is mediated by interaction with the Kelch-like ECH-associated protein 1 (Keap1)–Cullin3–Rbx1 complex, which mediates Nrf2 ubiquitination and subsequent proteasomal degradation [8].

The human Keap1 protein contains 27 cysteine resides, some of which act as sensors of oxidative stress. Oxidizing and electrophilic agents, such as free ROS and plant-derived phenolic compounds, among many others, can modify these cysteine residues [9]. This causes a conformational change in Keap1, preventing subsequent binding of newly synthesized Nrf2, which promptly accumulates in the nucleus [10]. There, Nrf2 associates with small Maf proteins (sMaf) and binds to antioxidant responsive elements (ARE) in the promoters of its target genes. This process is essential for the assembly of the RNA polymerase machinery and the initiation of transcription [11]. What happens to Nrf2 next remains to be determined in more detail; however, Nrf2 can either be degraded in the nucleus via the β-TrCP–GSK3β axis or alternatively it may translocate back to the cytoplasm where it is swiftly degraded by Keap1 [12,13].

The NF-κB cellular function and regulation

Nuclear factor-κB (NF-κB) is a family of transcription factors that includes RelA (p65), RelB, c-rel, p50 and p52 [14]. The NF-κB complex is a key transcription factor that mediates immune responses to bacterial and viral infections, inflammation, aspects of development, cell proliferation and protection against UV radiation [15]. Pro-inflammatory cytokines such as tumour necrosis factor (TNF)α, interleukin (IL)-1β and bacterial lipopolysaccharide (LPS) are among the most potent NF-κB activators, acting on the extracellular receptors and initiating a relay of intracellular phosphorylation events, which co-ordinate signalling and conditional cell responses [16]. Phosphorylation of IκBα, the negative regulator of NF-κB, prompts an interaction with the β-TrCP–Skp1–Cullin1 complex driving IκBα ubiqitination and proteasomal degradation, releasing NF-κB subunits,

Key words: haeme oxygenase-1 (HO-1), inhibitor of kappa light polypeptide gene enhancer in B-cells kinase beta (IKKβ), Kelch-like ECH-associated protein 1 (Keap1), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), nuclear factor-κB (NF-κB), neurodegeneration.

Abbreviations: AML, acute myeloid leukaemia; ARE, antioxidant response element; CBP, CREB-binding protein; CNS, central nervous system; HDAC, histone deacetylase; HO-1, haeme oxygenase-1; HSP90, heat shock protein 90; IKKβ, IkB kinase β; IL-1β, interleukin 1β; iNOS, inducible nitric oxide synthase; IkBα, NF-κB inhibitor α; KO, knockout; LPS, lipopolysaccharide; MS, multiple sclerosis; NF-κB, nuclear factor-κB; NQO1, NAD(P)H dehydrogenase quinone 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; RAC1, Ras-related C3 botulinum toxin substrate 1; ROS, reactive oxygen species; SFP, sulforaphane; TNFα, tumour necrosis factor α.

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which then translocate to the nucleus [17]. NF-κB homo- and hetero-dimers associate specifically with κB regulatory DNA consensus sequences upstream of NF-κB target genes, including IκBα an early-transcribed gene, which chaperones NF-κB back to the cytoplasm [18].

In order to respond effectively to acute inflammation, NF-κB also prompts an increase in mitochondrial activity and NADPH oxidase expression, which are the main sources of the endogenous free radicals [19,20].

It is now clear that robust NF-κB and Nrf2 activity is essential for maintaining co-ordinated cellular responses to resolve the inflammatory status of the cell/tissue. Imbalance between Nrf2 and NF-κB pathways is associated with a significant number of diseases ranging from neurodegeneration, autoimmune disorders and cancer [21]. It is well recognized that the Nrf2 and NF-κB pathways must interplay through multiple molecular interactions, the complexity and consequences of which are discussed below.

The interplay between Nrf2 and NF-κB pathways

The first insight into the interconnected nature of Nrf2 and NF-κB pathways came from the study of the Nrf2 knockout (KO) mice, which exhibit a neurodegenerative phenotype. Additionally, in the experimental model of brain injury the lack of Nrf2 has been associated with the augmentation of cytokine production [22]. Moreover, LPS-treated astrocytic and microglial cultures show more pronounced NF-κB activity compared with wild-type cells. The resulting elevation of cytokine production contributes to astrogliosis, neuronal death and demyelination of neuronal axons, which is an underlying cause of the neurodegenerative phenotype of the Nrf2 KO animals [23,24]. Also, studies on Nrf2$^{-/-}$ MEFs revealed enhanced IKKβ activity, augmenting the phosphorylation of IκBα and its subsequent degradation [25]. Both Nrf2 and NF-κB are regulated by redox sensitive factors and the absence of Nrf2 is associated with increased oxidative and nitrosative stress, leading to amplification of cytokine production, as NF-κB is more readily activated in oxidative environments [26].

There have been numerous studies conducted on Nrf2 activating phytochemicals such as sulforaphane (SFP), which is abundant in cruciferous vegetables, as well as synthetic inducers such as 2-cyano-3,12 dioxooleana-1,9 dien-28-imidazolide (CDDO-Im) and their anti-inflammatory potential. Pre-stimulation of Nrf2 in primary peritoneal macrophages dampens the production of COX-2 (cyclooxygenase 2), TNFα, iNOS (inducible nitric oxide synthase) and IL-1β in response to LPS. This effect has been attributed to Nrf2 activation, as the Nrf2$^{-/-}$ macrophages do not exhibit this anti-inflammatory capacity [27]. What is more, the increase inNrf2 activity in lupus nephritis leads to the accumulation of glutathione (GSH), which effectively buffers free radicals and prevents the activation of p65, resulting in reduced deposition of extracellular matrix [28]. Levels of GSH are thought to decrease with age and it is a likely contributing factor to the exacerbation of diseases underlined by chronic inflammation [29].

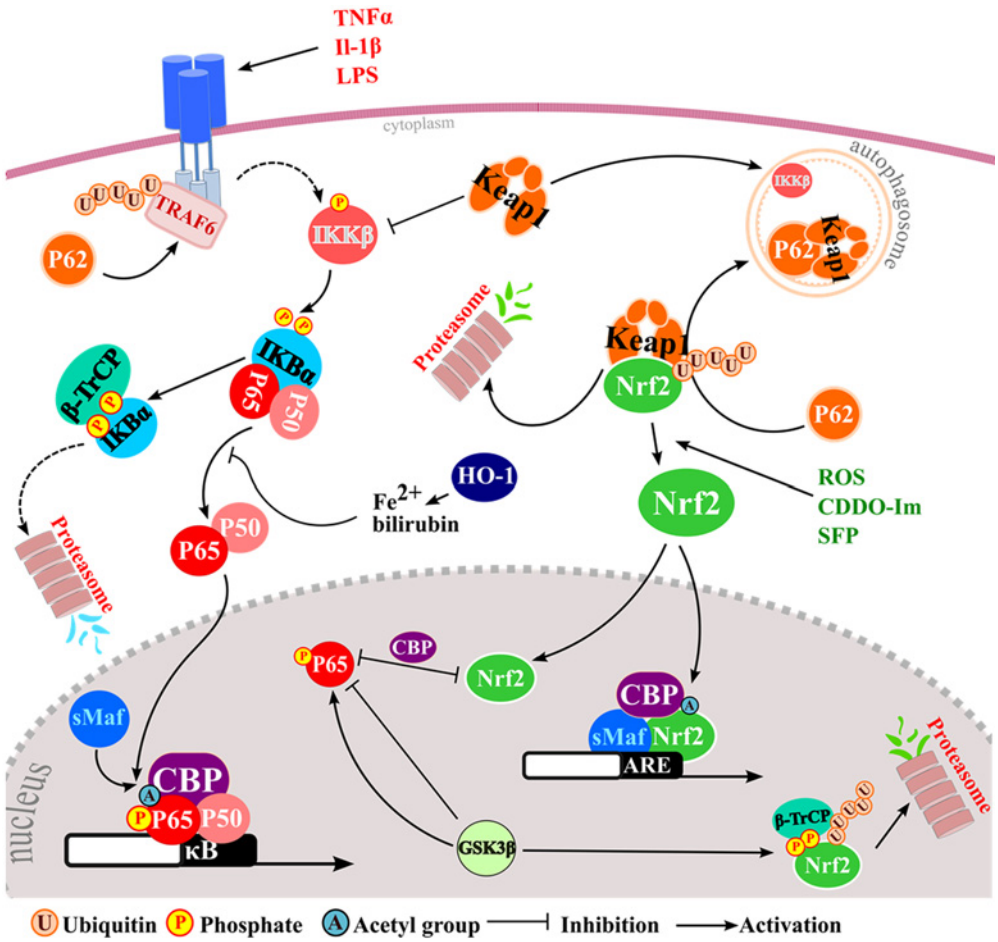
HO-1 is an Nrf2 target gene, which is at the core of Nrf2-mediated NF-κB inhibition. This enzyme is involved in haeme metabolism by catalysing the cleavage of the porphyrin ring of haeme into Fe^{2+}, carbon monoxide and biliverdin, which is consequently converted into bilirubin. Increases in HO-1 activity in endothelial cells leads to inhibition of NF-κB-mediated transcription of adhesion molecules such as E-Selectin and vascular cell adhesion molecule 1 (VCAM-1), through the action of bilirubin and possibly by the decrease in free intracellular iron ions [30]. A summary of known points of molecular cross-talk between Nrf2 and NF-κB is presented in Figure 1.

P65 and its role in modulating Nrf2 activity

The anti-inflammatory role of Nrf2 is well established; however, it should be noted that NF-κB activity also regulates Nrf2-mediated ARE expression. The modulatory action of NF-κB activation is somewhat complex and appears to be cell type dependent. There are several mechanisms by which p65 (the canonical NF-κB subunit) can exert a negative effect on ARE-linked gene expression. Work by Yu et al. [31] has shown that p65 assists in increasing the abundance of nuclear Keap1 levels. The localization of Keap1 is thought to be mostly cytoplasmic, however the authors demonstrated that in cells overexpressing p65, Keap1 diminished Nrf2–ARE signalling by translocating to the nucleus [31]. As the nuclear envelope does not permit free entry of proteins larger than 40 kDa, Keap1 is thought to enter the nucleus by interactions with the import facilitating karyopherin alpha 6 (importin alpha 7) (otherwise known as KPNA6) [32]. Consequently, KPNA6 overexpression results in decreased levels of HO-1 and NQO1. However, the ability of Keap1 to enter nuclear space and its potential nuclear functions are not well defined and remain to be studied in more detail.

The most well-established mechanism of inhibition of Nrf2 by p65 activity is a competition of both proteins for the transcriptional co-activator CBP (CREB-binding protein)–p300 complex. The CBP–p300 has an intrinsic histone acetyl transferase activity, which leads to local acetylation of histones and the loosening of chromatin structure, exposing DNA for transcriptional apparatus assembly. Aside from its role in histone modifications, it also acetylates non-histone proteins, with Nrf2 and p65 being well-defined targets [33,34]. The lysine residues in both transcription factors are subjected to acetyl group addition, which is thought to augment assembly of the transcriptional machinery and to enhance gene transcription [35]. CBP has been shown to interact with the Neh4 and Neh5 domains within Nrf2, which leads to the acetylation of the Neh1 domain, the DNA-binding portion of Nrf2. On the other hand, CBP also displays preference of binding to phosphorylated p65 at Ser^{276}. Overexpression of p65 is thought to limit the availability of CBP for Nrf2 complex formation, prioritizing transcription of the κB driven genes.

Figure 1 | Well-characterized points of molecular cross-talk between NF-κB and Nrf2 response pathways



Consequently, knockdown of p65 promotes the Nrf2–CBP association [34]. Nrf2 reciprocates this competition and the application of ethyl pyruvate on BV2 cells was shown to increase Nrf2 binding to CBP, thus depriving its interaction with p65, thereby further decreasing expression of NF-κB target genes such as iNOS [36].

In addition, p65 can induce repression of transcription by de-acetylation of histones, through association with histone deacetylase (HDAC). Liu et al. [34] also demonstrated that aside from competitive binding to CBP, p65 can promote HDAC3 association with MafK, thus preventing heterodimer formation with Nrf2 and therefore decreasing expression of ARE-related genes.

As stated previously, p65 is currently thought to have a dual role in the regulation of Nrf2 activity. Specific cell types show induction of Nrf2 protein levels and increased target gene expression in response to TNFα. Rushworth et al. [37] demonstrated that Nrf2 contains several κB sites in its proximal promoter, which are subject to binding and transcription initiation by p65. This underlies high basal Nrf2 activity in AML (acute myeloid leukaemia) cells and is believed to be the prime cause of chemoresistance of AML cells to the treatment with bortezomib [37].

The modulation of Nrf2 in response to NF-κB activation can act as a protective mechanism against the consequences of inflammation. Cuadrado et al. [38] established that the small GTPase RAC1 (Ras-related C3 botulinum toxin substrate 1) plays a key role in this process. RAC1 activation by LPS can activate Nrf2-mediated HO-1 expression, which in turn dampens the pro-inflammatory activity of NF-κB. RAC1 can also cause an NF-κB-mediated increase in Nrf2 levels, which is necessary for the resolution of the NF-κB activity, shifting the redox balance of the cell towards a more reducing environment [38].

Protein interactors linking the NF-κB and Nrf2 pathway

Aside from its regulatory role in the Nrf2–ARE pathway, Keap1 has been found to interact and negatively regulate IKKβ. IKKβ is targeted for degradation through autophagy in the absence of HSP90 (heat shock protein 90), which is a chaperone protein assisting in protein folding. Keap1 is thought to prevent HSP90 binding to IKKβ, which triggers its autophagic degradation. Additionally, Keap1 decreases the

phosphorylation of IKKβ possibly by concealing the residues to which phosphate groups are otherwise attached. Overall, the outcome of the Keap1–IKKβ interaction is the negative regulation of NF-κB though stabilization of IKBα [39].

Keap1 is thought to deplete Nrf2 from the cytoplasm, however the F-box protein β-TrCP, a component of the Skp1–Cullin1–β-TrCP E3 ligase complex, controls nuclear Nrf2 levels [12,40]. The mechanism of degradation via β-TrCP differs considerably from the Keap1 mode of action as it only recognizes and binds to phosphorylated substrates. The kinase that phosphorylates and marks Nrf2 for β-TrCP binding is GSK3β. Interestingly, p65 is also a substrate for GSK3β phosphorylation, which is thought to modulate p65 DNA binding affinity, but can have both positive and negative effects on NF-κB depending on the cellular context [41,42].

The canonical role of β-TrCP is the regulation of IκBα degradation in response to cytokines [17]. Therefore, β-TrCP function can lead to augmentation of NF-κB activity as well as to inhibition of Nrf2–ARE transcription.

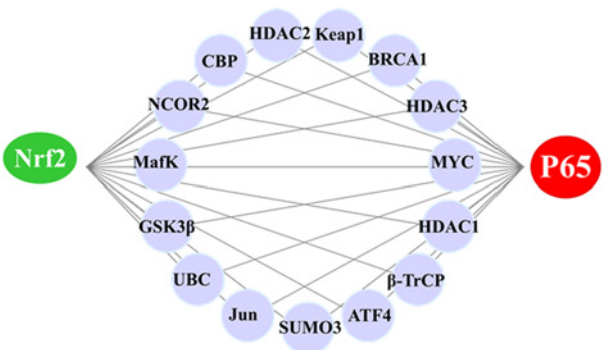
Another Nrf2 target gene, p62 is involved in modulation of antioxidant and inflammatory activities. p62 also acts as a protein scaffold, enhancing Nrf2 activity by mediating the autophagosomal degradation of Keap1 [43]. The ability of p62 to oligomerize, promotes ubiquitination and activation of TNFα receptor-associated factor 6 (TRAF6), enhancing nerve growth factor (NGF)-mediated NF-κB signalling [44].

Finally, Nrf2 is thought to act mainly as a dimer with sMaf proteins, one of them being MafK has been shown to modulate transcriptional activity of p65. MafK is thought to facilitate the interaction of p65 and CBP, leading to increases in acetylation of p65 and its overexpression augments production of cytokines in response to LPS. Knockdown of Nrf2 is associated with increased levels of MafK, therefore the activity of Nrf2 could be involved in maintaining low levels of this protein, disallowing excessive p65 acetylation [45].

Other interactors of Nrf2 and NF-κB components are presented in Figure 2. However, in general, their role in modulating the balance between the two pathways remains to be further explored.

Figure 2 | Common interactions between p65 and Nrf2

Data obtained from BioGRID were filtered to remove those identified only by co-localization studies or genetic interactions. Proteins are represented as coloured nodes with edges representing known protein interactions.



The importance of NF-κB and Nrf2 as targets in neurodegenerative diseases

Intensive research into anti-inflammatory properties of Nrf2 have identified it as a promising target in treating several neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and Parkinson's disease (PD). Several triterpenoids and phytochemical Nrf2-inducing compounds, such as CDDO-Im and SFP have shown promising results in reducing inflammation of the central nervous system (CNS) and importantly are also able to cross the blood-brain-barrier [24,46,47]. Drugs currently used in treating neurodegenerative disorders act primarily by regulating neuronal physiology through modulation of neurotransmitter release or by acting on neuronal receptors [48]. Importantly, Nrf2 has the potential to reduce numbers of overactive microglia and astrocytes, which are thought to make substantial contributors to CNS pathology [46,48,49]. In fact, the first drug approved by the U.S. Food and Drug Administration (FDA) in treatment of MS Tecfidera® (dimethyl fumarate) is likely to act primarily through transcriptional activation of Nrf2 target genes and has a potent anti-inflammatory action [50].

In addition to synthetic compounds that are used in disease settings, many phytochemicals naturally occurring in fruit and vegetables such as SFP, cinnamate, resveratrol or curcumin all have the potential to extenuate chronic inflammation. These compounds act by activating Nrf2 and increasing the antioxidant protection of the cells, to alleviate the damage from ROS [3,24,48]. Consuming Nrf2 activating compounds in food could therefore have a profound role in disease prevention.

Conclusions

Nrf2 and NF-κB are key pathways regulating the fine balance of cellular redox status and responses to stress and inflammation. The interplay between these pathways occurs through a range of complex molecular interactions and can often depend on the cell type and tissue context. These interactions operate through both transcriptional and post-transcriptional mechanisms, allowing fine-tuning of dynamic responses to ever-changing environmental cues. Despite convincing evidence for functional interactions between the Nrf2 and NF-κB pathways, many aspects of the conditional and dynamic nature of cross-talk remain unknown. As such, many important aspects of co-regulation, negative feedback and competitive binding are yet to be defined. Systematic investigation of protein complex composition and network analysis will provide new insights to drive development of rationally improved strategies to manipulate the balance between Nrf2 and NF-κB responses under both physiological and disease conditions.

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References

1 Kobayashi, A., Ohta, T. and Yamamoto, M. (2004) Unique function of the Nrf2–Keap1 pathway in the inducible expression of antioxidant and detoxifying enzymes. Methods Enzymol. **378**, 273–286 CrossRef PubMed
2 Dhakshinamoorthy, S. and Jaiswal, A.K. (2001) Functional characterization and role of INrf2 in antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. Oncogene **20**, 3906–3917 CrossRef PubMed
3 Thimmulappa, R.K., Mai, K.H., Srisuma, S., Kensler, T.W., Yamamoto, M. and Biswal, S. (2002) Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res. **62**, 5196–5203 PubMed
4 Lee, J.M., Calkins, M.J., Chan, K., Kan, Y.W. and Johnson, J.A. (2003) Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J. Biol. Chem. **278**, 12029–12038 CrossRef PubMed
5 Jain, A., Lamark, T., Sjøttem, E., Larsen, K.B., Awuh, J.A., Øvervatn, A., McMahon, M., Hayes, J.D. and Johansen, T. (2010) p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. J. Biol. Chem. **285**, 22576–22591 CrossRef PubMed
6 Kwak, M.K., Wakabayashi, N., Greenlaw, J.L., Yamamoto, M. and Kensler, T.W. (2003) Antioxidants enhance mammalian proteasome expression through the Keap1–Nrf2 signaling pathway. Mol. Cell. Biol. **23**, 8786–8794 CrossRef PubMed
7 Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., Yamamoto, M. and Motohashi, H. (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell **22**, 66–79 CrossRef PubMed
8 Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D. and Yamamoto, M. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. **13**, 76–86 CrossRef PubMed
9 Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M. and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc. Natl. Acad. Sci. U.S.A. **18**, 11908–11913 CrossRef
10 Kansanen, E., Kuosmanen, S.M., Leinonen, H. and Levonen, A.L. (2013) The Keap1–Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. **1**, 45–49 CrossRef PubMed
11 Hirotsu, Y., Katsuoka, F., Funayama, R., Nagashima, T., Nishida, Y., Nakayama, K., Engel, J.D. and Yamamoto, M. (2012) Nrf2–MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res. **40**, 10228–10239 CrossRef PubMed
12 Rada, P., Rojo, A.I., Chowdhry, S., McMahon, M., Hayes, J.D. and Cuadrado, A. (2011) SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol. Cell. Biol. **31**, 1121–1133 CrossRef PubMed
13 Niture, S.K., Jain, A.K., Shelton, P.M. and Jaiswal, A.K. (2011) Src subfamily kinases regulate nuclear export and degradation of transcription factor Nrf2 to switch off Nrf2-mediated antioxidant activation of cytoprotective gene expression. J. Biol. Chem. **286**, 28821–28832 CrossRef PubMed
14 Baldwin, Jr, A.S. (1996) The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu. Rev. Immunol. **14**, 649–683 CrossRef PubMed
15 Smale, S.T. (2011) Hierarchies of NF-kappaB target-gene regulation. Nat. Immunol. **12**, 689–694 CrossRef PubMed
16 Sakurai, H., Suzuki, S., Kawasaki, N., Nakano, H., Okazaki, T., Chino, A., Doi, T. and Saiki, I. (2003) Tumor necrosis factor-alpha-induced IKK phosphorylation of NF-kappaB p65 on serine 536 is mediated through the TRAF2, TRAF5, and TAK1 signaling pathway. J. Biol. Chem. **278**, 36916–36923 CrossRef PubMed
17 Winston, J.T., Strack, P., Beer-Romero, P., Chu, C.Y., Elledge, S.J. and Harper, J.W. (1999) The SCFbeta–TRCP–ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. Genes Dev. **13**, 270–283 CrossRef PubMed
18 Sen, R. and Baltimore, D. (1986) Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell **47**, 921–928 CrossRef PubMed
19 Manea, A., Manea, S.A., Gafencu, A.V. and Raicu, M. (2007) Regulation of NADPH oxidase subunit p22(phox) by NF-kB in human aortic smooth muscle cells. Arch. Physiol. Biochem. **113**, 163–172 CrossRef PubMed
20 Mauro, C., Leow, S.C., Anso, E., Rocha, S., Thotakura, A.K., Tornatore, L., Moretti, M., De Smaele, E., Beg, A.A., Tergaonkar, V. et al. (2011) NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. Nat. Cell Biol. **13**, 1272–1279 CrossRef PubMed
21 Ben-Neriah, Y. and Karin, M. (2011) Inflammation meets cancer, with NF-kappaB as the matchmaker. Nat. Immunol. **12**, 715–723 CrossRef PubMed
22 Pan, H., Wang, H., Wang, X., Zhu, L. and Mao, L. (2012) The absence of Nrf2 enhances NF-kappaB-dependent inflammation following scratch injury in mouse primary cultured astrocytes. Mediators Inflamm. **2012**, 217580 CrossRef PubMed
23 Frakes, A.E., Ferraiuolo, L., Haidet-Phillips, A.M., Schmelzer, L., Braun, L., Miranda, C.J., Ladner, K.J., Bevan, A.K., Foust, K.D., Godbout, J.P. et al. (2014) Microglia induce motor neuron death via the classical NF-kappaB pathway in amyotrophic lateral sclerosis. Neuron **81**, 1009–1023 CrossRef PubMed
24 Neymotin, A., Calingasan, N.Y., Wille, E., Naseri, N., Petri, S., Damiano, M., Liby, K.T., Risingsong, R., Sporn, M., Beal, M.F. and Kiaei, M. (2011) Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free Radic. Biol. Med. **51**, 88–96 CrossRef PubMed
25 Thimmulappa, R.K., Lee, H., Rangasamy, T., Reddy, S.P., Yamamoto, M., Kensler, T.W. and Biswal, S. (2006) Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J. Clin. Invest. **116**, 984–995 CrossRef PubMed
26 Ganesh Yerra, V., Negi, G., Sharma, S.S. and Kumar, A. (2013) Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-kappaB pathways in diabetic neuropathy. Redox Biol. **1**, 394–397 CrossRef PubMed
27 Lin, W., Wu, R.T., Wu, T., Khor, T.O., Wang, H. and Kong, A.N. (2008) Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. Biochem. Pharmacol. **76**, 967–973 CrossRef PubMed
28 Jiang, T., Tian, F., Zheng, H.T., Whitman, S.A., Lin, Y.F., Zhang, Z.G., Zhang, N. and Zhang, D.D. (2014) Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-kappa B-mediated inflammatory response. Kidney Int. **85**, 333–343 CrossRef PubMed
29 Suh, J.H., Shenvi, S.V., Dixon, B.M., Liu, H., Jaiswal, A.K., Liu, R.M. and Hagen, T.M. (2004) Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proc. Natl. Acad. Sci. U.S.A. **101**, 3381–3386 CrossRef PubMed
30 Soares, M.P., Seldon, M.P., Gregoire, I.P., Vassilevskaia, T., Berberat, P.O., Yu, J., Tsui, T.Y. and Bach, F.H. (2004) Heme oxygenase-1 modulates the expression of adhesion molecules associated with endothelial cell activation. J. Immunol. **172**, 3553–3563 CrossRef PubMed
31 Yu, M.A., Li, H., Liu, Q.M., Liu, F., Tang, L.J., Li, C.Y., Yuan, Y.Z., Zhan, Y.Q., Xu, W.X., Li, W. et al. (2011) Nuclear factor p65 interacts with Keap1 to repress the Nrf2–ARE pathway. Cell. Signal. **23**, 883–892 CrossRef PubMed
32 Sun, Z., Wu, T.D., Zhao, F., Lau, A., Birch, C.M. and Zhang, D.D. (2011) KPNA6 (Importin alpha 7)-mediated nuclear import of Keap1 represses the Nrf2-dependent antioxidant response. Mol. Cell. Biol. **31**, 1800–1811 CrossRef PubMed
33 Sun, Z., Chin, Y.E. and Zhang, D.D. (2009) Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol. Cell. Biol. **29**, 2658–2672 CrossRef PubMed
34 Liu, G.H., Qu, J. and Shen, X. (2008) NF-kappaB/p65 antagonizes Nrf2–ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. Biochim. Biophys. Acta **1783**, 713–727 CrossRef PubMed
35 Wang, B., Zhu, X.L., Kim, Y., Li, J., Huang, S.Y., Saleem, S., Li, R.C., Xu, Y., Dore, S. and Cao, W.S. (2012) Histone deacetylase inhibition activates transcription factor Nrf2 and protects against cerebral ischemic damage. Free Radic. Biol. Med. **52**, 928–936 CrossRef PubMed

36 Kim, S.W., Lee, H.K., Shin, J.H. and Lee, J.K. (2013) Up-down regulation of HO-1 and iNOS gene expressions by ethyl pyruvate via recruiting p300 to Nrf2 and depriving it from p65. Free Radic. Biol. Med. **65**, 468–476 CrossRef PubMed
37 Rushworth, S.A., Zaitseva, L., Murray, M.Y., Shah, N.M., Bowles, K.M. and MacEwan, D.J. (2012) The high Nrf2 expression in human acute myeloid leukemia is driven by NF-kappaB and underlies its chemo-resistance. Blood **120**, 5188–5198 CrossRef PubMed
38 Cuadrado, A., Martin-Moldes, Z., Ye, J. and Lastres-Becker, I. (2014) Transcription factors NRF2 and NF-kappaB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. J. Biol. Chem. **289**, 15244–15258 CrossRef PubMed
39 Kim, J.E., You, D.J., Lee, C., Ahn, C., Seong, J.Y. and Hwang, J.I. (2010) Suppression of NF-kappaB signaling by KEAP1 regulation of IKKbeta activity through autophagic degradation and inhibition of phosphorylation. Cell. Signal. **22**, 1645–1654 CrossRef PubMed
40 Chowdhry, S., Zhang, Y., McMahon, M., Sutherland, C., Cuadrado, A. and Hayes, J.D. (2013) Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene **32**, 3765–3781 CrossRef PubMed
41 Park, S.H., Park-Min, K.H., Chen, J., Hu, X. and Ivashkiv, L.B. (2011) Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. Nat. Immunol. **12**, 607–615 CrossRef PubMed
42 Lee, J. and Kim, M.S. (2007) The role of GSK3 in glucose homeostasis and the development of insulin resistance. Diabetes Res. Clin. Pract. **77** (Suppl 1), S49–S57 CrossRef PubMed
43 Ichimura, Y., Waguri, S., Sou, Y., Kageyama, S., Hasegawa, J., Ishimura, R., Saito, T., Yang, Y.J., Kouno, T., Fukutomi, T. et al. (2013) Phosphorylation of p62 activates the Keap1–Nrf2 pathway during selective autophagy. Mol. Cell **51**, 618–631 CrossRef PubMed
44 Wooten, M.W., Geetha, T., Seibenhener, M.L., Babu, J.R., Diaz-Meco, M.T. and Moscat, J. (2005) The p62 scaffold regulates nerve growth factor-induced NF-kappaB activation by influencing TRAF6 polyubiquitination. J. Biol. Chem. **280**, 35625–35629 CrossRef PubMed
45 Hwang, Y.J., Lee, E.W., Song, J., Kim, H.R., Jun, Y.C. and Hwang, K.A. (2013) MafK positively regulates NF-kappaB activity by enhancing CBP-mediated p65 acetylation. Scientific Rep. **3**, 3242
46 Brandenburg, L.O., Kipp, M., Lucius, R., Pufe, T. and Wruck, C.J. (2010) Sulforaphane suppresses LPS-induced inflammation in primary rat microglia. Inflamm. Res. **59**, 443–450 CrossRef PubMed
47 Danilov, C.A., Chandrasekaran, K., Racz, J., Soane, L., Zielke, C. and Fiskum, G. (2009) Sulforaphane protects astrocytes against oxidative stress and delayed death caused by oxygen and glucose deprivation. Glia **57**, 645–656 CrossRef PubMed
48 Calkins, M.J., Johnson, D.A., Townsend, J.A., Vargas, M.R., Dowell, J.A., Williamson, T.P., Kraft, A.D., Lee, J.-M., Li, J. and Johnson, J.A. (2009) The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease. Antioxid. Redox Signal. **11**, 497–508 CrossRef PubMed
49 Innamorato, N.G., Rojo, A.I., Garcia-Yague, A.J., Yamamoto, M., de Ceballos, M.L. and Cuadrado, A. (2008) The transcription factor Nrf2 is a therapeutic target against brain inflammation. J. Immunol. **181**, 680–689 CrossRef PubMed
50 Jing, X., Shi, H., Zhang, C., Ren, M., Han, M., Wei, X., Zhang, X. and Lou, H. (2014) Dimethyl fumarate attenuates 6-OHDA-induced neurotoxicity in SH-SY5Y cells and in animal model of Parkinson's disease by enhancing Nrf2 activity. Neuroscience **286**, 131–140 CrossRef PubMed

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