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Review

NFE2L2 (nuclear factor, erythroid 2-like 2)

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Abstract

Review on NFE2L2, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: NRF2

HGNC (Hugo): NFE2L2

Location: 2q31.2

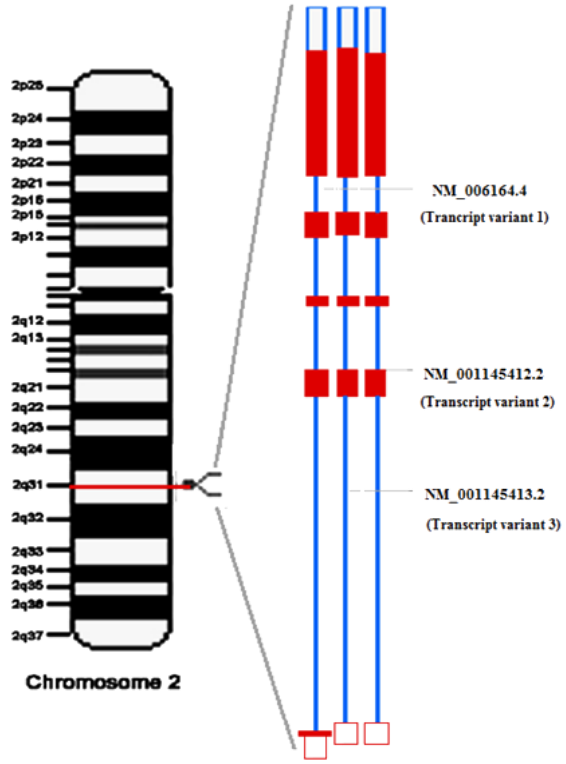
Note

The transcription factor Nrf2, encoded by the NFE2L2 gene, is the mediator of a cellular antioxidant response. Nrf2 belongs to the cap'n'collar (cnc) family of transcription factors.

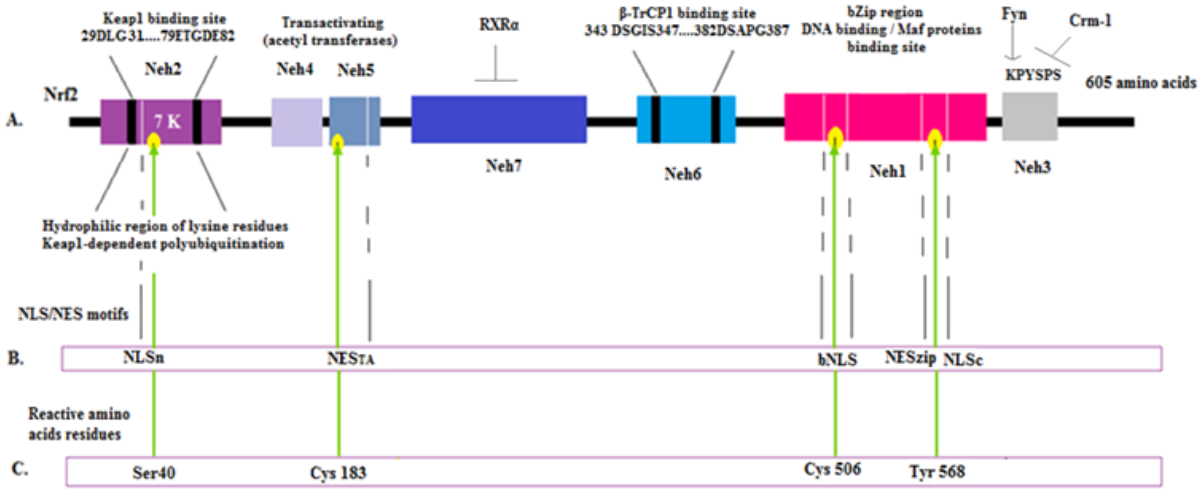
DNA/RNA

Description

The NFE2L2 gene is approximately 34.8 kb in size. The mouse homologue of the NFE2L2 gene is a self- and hetero-inducible gene; its promoter region contains two ARE/EpRE (antioxidant or electrophile response element) sequences at -492 bp and -754 bp, through which it is induced by Nrf2. In addition, its regulatory region contains three XRE (xenobiotic response element) sequences at -712 bp, +755 bp and +850 bp. ARE and XRE sequences are implicated in the inducible upregulation of the gene¡s transcription. A kB2 region for NF-κB binding has been detected at +270 bp; proinflammatory stimuli can induce human NFE2L2 transcription via this element. On the other hand, five CpG sequences in the promoter region of NFE2L2 allow hypermethylation and repression of gene expression (Kwak et al., 2002; Hayes and Dinkova-Kostova, 2014).



NFE2L2 gene - transcript variants. NFE2L2 gene located on chromosome 2. Multiple transcript variants (TV) encoding different isoforms have been found for this gene. The transcript variants referenced more often in the literature are NM_006164.4 (TV 1), NM_001145412.2 (TV 2) and NM_001145413.2 (TV 3).



Structural and functional domains of Nrf2. A. Structural features of the human Nrf2 protein that dictate its activity. The interaction with Keap1 is mediated by the Neh2 (Nrf2 extended homology 2) domain of Nrf2. **B.** Nuclear localisation signals (NLS) and nuclear export signals (NES) have been detected in the Nrf2 sequence. The NESTA motif is the only one shown to be directly redox-sensitive. **C.** Specific cysteine residues have been characterised as reactive, meaning that they are sensitive to oxidation. Oxidative stress also activates intracellular kinases such as PKC and Fyn which in turn phosphorylate Nrf2 (on Ser40 and Tyr568, respectively) contributing to Nrf2 activation (Jain et al., 2005; Zhang, 2006).

Transcription

NM_006164.4 (TV 1) comprises 5 coding exons and is approximately 2.8 kb in size; this transcript encodes the longest protein isoform. NM_001145412.2 (TV 2) and NM_001145413.2 (TV 3) comprise 4 coding exons each and are approximately 2.7 kb and 2.4 kb in size, respectively.

MiRNAs

miRNA3128 is a non-coding RNA that is the transcript product of a region in the first intron of NFE2L2 (chr. 2: 177255945-177256010, complement). It is not known whether it has a role in the regulation of NFE2L2 expression. Micro RNAs species reported to suppress NFE2L2 expression include miR-27a, miR-28, miR-34a, miR-93, miR-142-5p, miR-144, and miR-153 (Filipowicz et al., 2008; Cheng et al., 2013; Hayes and Dinkova-Kostova, 2014).

Protein

Note

Name: Nuclear factor erythroid 2-related factor 2. Short: NF-E2-related factor 2, NFE2-related factor 2.

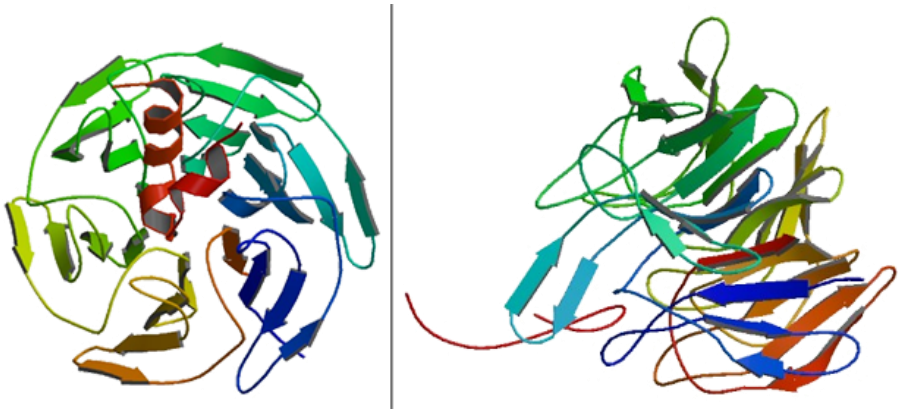
Alternatives names: Nuclear factor, erythroid derived 2, like 2, NF-E2 p45-related Factor-2.

Nrf2, the nuclear factor erythroid 2 (NF-E2)-related transcription factor 2, was first described in 1994 by Moi et al. by screening for factors that could bind to a NFE2-binding DNA sequence. The human Nrf2 protein NP_006155.2 (encoded by TV 1) is 67.8 kDa in weight and consists of 605 amino acids; NP_001138884.1 (encoded by TV2) is 66.1 kDa in weight and consists of 589 amino acids; and NP_001138885.1 (encoded by TV3) is 65.4 kDa in size and consists of 582 amino acids.

Nrf2 has been characterized as a modulator protein and is the core of the Nrf2 antioxidant system/pathway. Other main components of the pathway are Keap1, the negative regulator of Nrf2, and small Maf proteins which serve as cofactors for Nrf2 binding to regulatory DNA sequences of ARE-regulated genes (Moi et al., 1994; Li and Kong, 2009).

Description

Nrf2 binds to ARE sites of antioxidant genes as a heterodimer. Specifically, Nrf2 heterodimerizes with small Maf proteins (which are themselves devoid of transcription activating domains) to induce the transcription of ARE-regulated genes. Other binding partners include members of the AP-1 transcription factor family like Jun and Fos. In contrast, homodimers or heterodimers of the different small Maf proteins, and heterodimers of Bach proteins with small Maf proteins on AREs, have been characterised as negative regulators of Nrf2 signalling pathway that compete with Nrf2 for binding to AREs. The co-factor CBP/p300 is indispensable for transcription activation by Nrf2 and localizes to ARE-binding sites in association with Nrf2-small Maf heterodimers (Dhakshinamoorthy et al., 2005; Ishikawa et al., 2005; Li et al., 2008; Hirotsu et al., 2012).



Crystal structures of the Nrf2-binding domain of Keap1 (Keap1 DC/Kelch domain) in complex with peptides derived from the Keap1-binding domain of Nrf2 (the DLG motif of the mouse Neh2 domain or the ETGE motif of the human Neh2 domain).

Expression

Nrf2 is considered to be ubiquitously expressed, as it has been shown to be expressed in various cell types (including lung, liver, kidney, stomach, small intestine, neurons, astrocytes, etc) and it has been considered a multi-organ protector that enhances the cellular resistance to potential harmful insults that occur during cells' normal activities and during environmental exposures. Morevoer, Nrf2 has been found to be overexpressed in various human cancers (Yoo et al., 2012; Lee and Surh, 2005).

Localisation

Nrf2 can be detected both in the cytoplasm and in the nucleus; the Keap1-mediated degradation of Nrf2 occurs in the cytoplasm. In addition, one of the proposed models for the Keap1-Nrf2 interaction suggests that a Keap1 dimer can bind one Nrf2 molecule and one PGAM5 molecule. PGAM5 possesses a N-terminal membrane targeting signal, through which the Nrf2-Keap1-PGAM complex is tethered to the cytosolic surface of the outer mitochondrial membrane. As a result, Nrf2 can be also localised in the perimitochondrial region (Sykiotis and Bohmann, 2010).

Function

The Nrf2/Keap1 pathway is a major mediator of cytoprotective responses to oxidative and electrophilic stress. Nrf2 responds to oxidative stress by inducing the transcriptional upregulation of a broad range of cytoprotective genes whose promoters contain Antioxidant Response Element (ARE) sequences. Specifically, Nrf2 translocates to the nucleus, heterodimerizes with small Maf proteins and binds to ARE sequences to induce gene transcription. When redox balance is restored, Nrf2 activity is repressed via export from the nucleus back into the cytoplasm and degradation via a Cullin - RING ligase 3 - Keap1 complex (CRLkeap1 complex), and by other mechanisms (Zhang, 2006).

Oxidative stress and the antioxidant transcriptional response mediated by Nrf2

Cells and tissues are constantly exposed to various oxidative substances and electrophilic chemicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), derived from both endogenous and exogenous sources. To adapt to the oxidative environment, cells have developed elaborate and highly efficient antioxidant machineries. When pro-oxidant and electrophilic challenges overwhelm the cell's antioxidant and detoxification proteins, cells experience oxidative stress. Oxidative stress conditions can cause damage to cellular structures, including lipids, proteins, and nucleic acids. Among other injuries, this can lead to mutations and epigenetic perturbations by damaging DNA and proteins that modify chromatin. Thus, oxidative stress can be a causative or exacerbating factor in a range of diseases, including, for example, respiratory and metabolic disorders, neurodegenerative diseases, and cancer.

In order to maintain homeostasis in the face of oxidative insults, cells possess signalling pathways that can sense oxidative stress and launch adaptive responses. Multiple ways of managing the intracellular oxidative load have been identified over the last two decades; among them, it has been recognized that gene transcription can be regulated by redox reactions. Prominent among the redox-sensitive pathways of gene activation is the Nrf2 system. The core of this pathway comprises the transcription factor Nrf2 and its negative regulator Keap1. In addition, small Maf proteins serve as dimerization partners of Nrf2 to facilitate its binding to DNA on special sequences termed antioxidant response elements (AREs) or
electrophile response elements (EpREs) in the regulatory regions of the many Nrf2-regulated genes, including the genes encoding glutamate cysteine ligase catalytic subunit (GCLC), heme

oxygenase-1 (HO-1), NADP(H) quinone oxidoreductase-1 (NQO1), microsomal glutathione-S-transferases such as MGST1 and MGST2, and multi-drug resistance-associated proteins such as ATP-binding cassette, subfamily C (CFTR/MRP), member 1 (ABCC1) (Magesh et al., 2012; Hayes and Dinkova-Kostova, 2014).

Activity and regulation of Nrf2

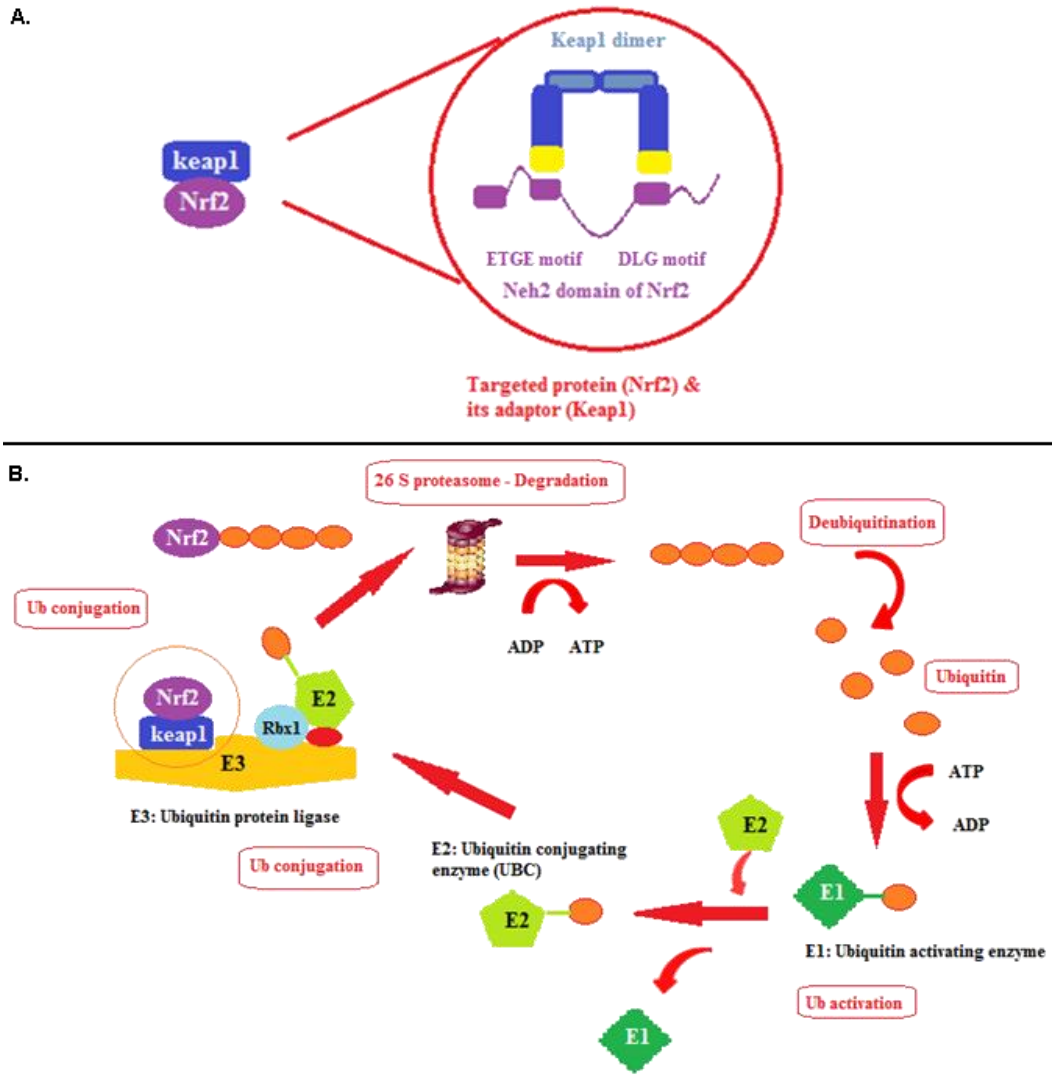
The Nrf2 pathway responds to oxidative stress by inducing the transcriptional upregulation of a broad range of cytoprotective genes.

The Nrf2 system responds to both endogenous reactive molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and to

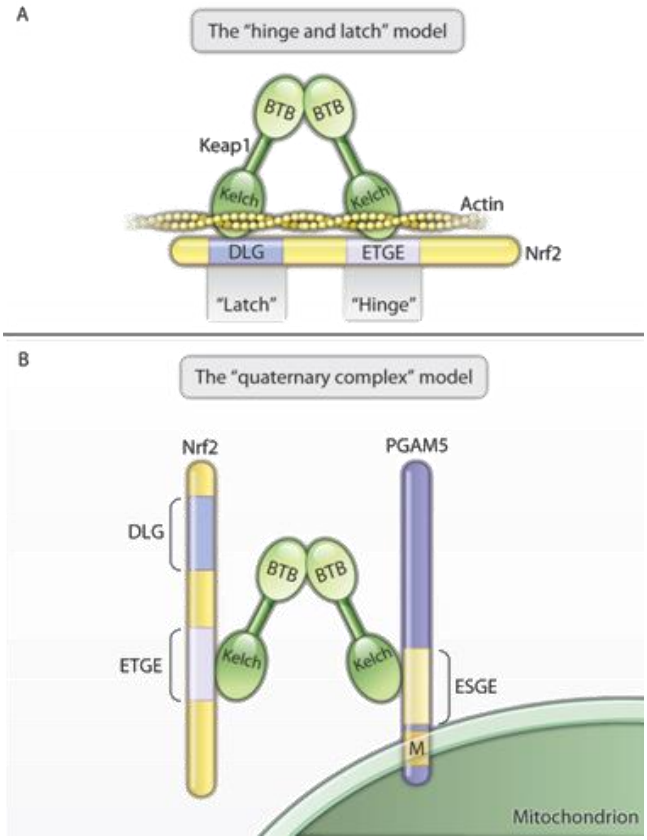
exogenous substances.

The sensing mechanisms comprise oxidation or alkylation of critical Keap1 cysteine residues (including Cys151, Cys273 and Cys288), and phosphorylation of Nrf2 on amino acids Ser40 and Tyr568 (Zhang and Hannink, 2003; Yamamoto et al., 2008; Magesh et al., 2012).

When redox homeostasis is restored, Nrf2 activity is repressed via export from the nucleus back into the cytoplasm and degradation via a Cullin-RING ligase 3 - Keap1 complex (CRLkeap1 complex). Nrf2 can also trigger a feedback loop of increased expression of ARE-dependent genes including Keap1 and Cul3, which then promote Nrf2 degradation and thus participate in resetting Nrf2 activity at its basal level (Rachakonda et al., 2008; Eggler et al., 2009; Baird and Dinkova-Kostova, 2011).



A. Keap1 binds Nrf2 and inhibits its transcriptional activity. The depicted "hinge-and-latch" model proposes a 2:1 Keap1:Nrf2 molecular stoichiometry. **B.** At the basal cellular state (low oxidative burden), Nrf2 undergoes Keap1-Cul3-mediated poly-ubiquitination and proteasomal degradation.



Two mechanistic models for the Nrf2-Keap1 interaction. Adapted from Sykiotis and Bohmann, 2010.

Activation of Nrf2 pathway

A. Transcriptional induction of the NFE2L2 gene

A1. Nrf2 autoregulation

It has been shown that the promoter of the mouse orthologue of the NFE2L2 gene includes two antioxidant response element-like sequences (ARE-L1 and ARE-L2), which are located at -492 bp and -754 bp from the transcription start site, respectively. It has been proposed that under stress conditions newly translated Nrf2 protein escapes Keap1-mediated degradation and binds to the ARE-L1 and ARE-L2 sequences to induce NFE2L2 gene transcription in a feed-forward manner (Shin et al., 2007; Hayes and Dinkova-Kostova, 2014).

A2. NFE2L2 transcription induced by the oncoproteins K-Ras and B-Raf

It has been demonstrated that oncogene-directed increased expression of the NFE2L2 gene can be an alternative mechanism of Nrf2 activation. K-Ras and B-Raf, which operate in the mitogen-activated protein kinase (MAPK) pathway, have been shown to increase NFE2L2 transcription via activation of Jun or/and Myc. It has been proposed that via this mechanism oncogenic signaling may modulate redox homeostasis during tumirogenesis (DeNicola et al., 2011).

A3. Cross-talk of Nrf2 with the NF-κB and AhR signaling pathways

In general, the effects of the intracellular events induced by Nrf2 activation lead to NF-κB suppression, and vice versa; thus, overall, Nrf2 signaling antagonises NF-κB signaling. Nevertheless, in acute myeloid leukemia it has been reported that Nrf2 is upregulated by NF-κB-mediated transactivation of the NFE2L2 gene by direct binding of NF-κB to the NFE2L2 promoter (Rushworth et al., 2012).

The AhR/ARNT complex (aryl hydrocarbon receptor / AhR nuclear translocator) regulates gene transcription in response to xenobiotics, such as polycyclic aromatic hydrocarbons, via binding to xenobiotic response elements (XREs). Three XRE-like elements have been identified in the mouse Nfe2l2 promoter; via these elements, the AhR/XRE pathway can control Nrf2/ARE signaling (Miao et al., 2005).

A4. BRCA1 / ARNT-mediated induction of NFE2L2 gene transcription

The transcription factor BRCA1 (breast cancer 1, early onset) has been reported to increase the transcription of the NFE2L2 gene. As BRCA1 has the ability to interact with ARNT, it is possible that BRCA1 induces Nrf2 expression in an ARNT-dependent manner (Kang et al., 2006).

B. Post-translational activation of Nrf2

B1. The "hinge and latch" and "quaternary" models

At basal conditions (meaning the absence of oxidative stress) the NFE2L2 gene is constantly transcribed and Nrf2 protein is constantly synthesized, but Nrf2 protein abundance and activity are maintained at low levels due to the negative regulation of Nrf2 by Keap1 through the CRLkeap1 complex.

The "hinge and latch" model has been proposed as a mechanistic model that accounts for the interaction between Nrf2 and Keap1 and provides as structural basis for the Keap1-dependent polyubiquitination and degradation of Nrf2. This model posits an interaction of one Nrf2 molecule with a Keap1 homodimer, in which the high affinity binding of the ETGE motif of the Neh2 domain of Nrf2 functions as a "hinge" to fix Nrf2 to one of two Keap1 molecules, whereas the low affinity binding of the DLG motif of the Neh2 domain of Nrf2 functions as a "latch" to lock down the Neh2 domain to the other Keap1 molecule of the homodimer. The fixation of the Neh2 domain between the two Keap1 molecules thus facilitates its ubiquitination and the subsequent degradation of Nrf2 by the 26S proteasome. A competing structural model is the "quaternary complex" model, which proposes that a Keap1 dimer binds two molecules of substrate through high-affinity interactions with ETGE motifs. Specifically, a Keap1 dimer can bind two Nrf2 molecules, or one Nrf2 molecule and one PGAM5 molecule. PGAM5 possesses an N-terminal membrane targeting signal through which the Nrf2-Keap1-PGAM complex is tethered to the cytosolic surface of the outer mitochondrial membrane (Tong et al., 2006; Sykiotis and Bohmann, 2010; Kansanen et al., 2012), potentially to allow Nrf2 to be activated in response to mitochondrial leakage of ROS.

Under conditions of oxidative stress, it is believed that the oxidative modification of certain cysteine residues of Keap1 leads to conformational changes of the Keap1 dimer. In the "hinge and latch" model, this results in dissociation of the DLG motif from Keap1, wuch that Nrf2 cannot be properly presented for ubiquitination by the CRL^{keap1} complex and thus escapes proteosomal degradation. The stabilized Nrf2 accumulates in the nucleus where it heterodimerizes with small Maf proteins and binds to AREs, leading to transcription of ARE-dependent cytoprotective genes (reviewed in Sykiotis and Bohmann, 2010; Hayes and Dinkova-Kostova, 2014).

B2. Phosphorylation of Nrf2 by PKC

Several protein kinases, including protein kinase C (PKC), have been implicated in the upstream regulation of Nrf2 pathway. Specifically, phosphorylation of Nrf2 by PKC induces nuclear translocation of this transcription factor and activation of the ARE in response to oxidative stress. Furthermore, it has been found that PKC phosphorylates Nrf2 at Ser-40 facilitating its release from Keap1-mediated inhibition (Wakabayashi et al., 2010; Stepkowski and Kruszewski, 2011).

B3. The redox signaling "model of two Nrf2 pools"

Multiple NLS/NES (Nuclear localisation signal/ nuclear export signal) motifs have been identified in the Nrf2 sequence. These include three NLS motifs (bNLS, NLS_N and NLS_c) and two NES motifs (NES_{TA} and NES_{ZIP}) but only the NESTA motif has been found to be redox-sensitive. Specifically, the NESTA motif has been shown to display a graded response to oxidative stress, implying that it can not only sense the presence of reactive oxidative species, but it also has the ability to transmit the oxidative stress "intensity" to the nucleus in order to up-regulate the transcription of ARE-genes accordingly. Based on these observations, Nrf2 has been proposed as a direct redox-sensor. Specifically, under basal condition a dynamic balance can be observed as the combined nuclear exporting forces of NES_{TA} and NES_{ZIP} counteract the combined nuclear importing force of the bNLS, NLS_N and NLS_c leading to a whole-cell distribution of Nrf2. However, under oxidative stress the NES_{TA} is functionally disabled, and the driving force of NLSs becomes dominant and favors the nuclear localization of Nrf2.

The NLS/NES motifs and their role in activation of Nrf2 have led to the hypothesis for Keap1-independent Nrf2 signaling. Nevertheless, this model does not exclude Keap1 involvement in redox signaling. Consequently, a new model has been proposed that encompasses both Keap1-dependent and Keap1-independent Nrf2 signaling. This model proposes that in cells there may exist a free-floating pool of Nrf2 (fNrf2) and a Keap1-bound pool of Nrf2 (kNrf2). Under homeostatic conditions there is an equilibrium between synthesis and degradation of Nrf2, such that the fNrf2 pool remains small. But when cells are exposed to oxidative stress, the Nrf2-binding capacity of Keap1 is diminished and the fNrf2 pool is enlarged. As the NESTA of the fNrf2 redox-sensitive pool is disabled by the stress, nuclear localization of Nrf2 is favored (Li and Kong, 2009).

B4. Competitors of Nrf2 for binding to Keap1

It has been demonstrated that the ability of Keap1 to repress Nrf2 can be modulated by proteins that also possess ETGE motifs and thereby compete with Nrf2 for the same binding site in Keap1. For example, dipeptidyl-peptidase 3 (DPP3), IκB kinase β (IKKβ), partner and localizer of BRCA2 (PALB2), phosphoglycerate mutase 5 (PGAM5) and Wilms tumor gene on X chromosome (WTX) contain ETGE motifs that enable them to bind Keap1 and act as competitors of Nrf2 (Hayes and Dinkova-Kostova, 2014).

B5. mTOR signaling and p62-dependent degradation of Keap1

There is also cross-talk between Nrf2-Keap1 signaling and autophagy. It has been shown that in normal cells this interaction serves as a host defence mechanism leading to expression of antioxidant enzymes as well as elimination of cytotoxic products. Keap1 can bind the autophagy cargo receptor p62, which contains an STGE motif similar to the ETGE motif of Nrf2. Following phosphorylation of Ser 351 within its STGE motif by the mammalian target of rapamycin complex (mTORC1), p62 becomes a potent inhibitor of Keap1. p62 is phosphorylated by mTORC1 in the presence of ubiquitinated autophagic cargos, which can occur under oxidative conditions; this in turn favors the binding of Keap1 to the phosphorylated STGE motifs. As a result, Keap1 is sequestrated in autophagy cargos in a p62-dependent manner, allowing Nrf2 to be stabilized and to accumulate in the nucleus to induce cytoprotective enzymes (Komatsu et al., 2010; Ichimura et al., 2013; Lamming and Sabatini, 2013).

B6. Cross-talk between p53/p21 and the Nrf2 pathway

The p53 tumor suppressor protein regulates several intracellular procedures including gene transcription and induction of apoptosis. It has been demonstrated that p53 is implicated in the regulation of the Nrf2-mediated oxidative response in a dual manner: under low or mild levels of oxidative stress, p53 promotes the stabilization of Nrf2 and its subsequent nuclear accumulation through the transcriptional activation of p21, and as a result reduces the oxidative burden to promote cell survival. p21 stabilizes Nrf2 due to the existence of a KRR motif within the p21 sequence which interacts with the DLG motif of Nrf2 inhibiting its binding to Keap1. On the other hand, under conditions of high or sustained levels of oxidative stress, Nrf2-mediated cell survival is suppressed, and high activity levels of p53 induce apoptosis to prevent tumorigenesis (Chen et al., 2009; Chen W et al., 2012).

B7. Competitive binding of BRCA1 to Nrf2

The ability of Keap1 to repress Nrf2 can be diminished by the competitive binding of breast cancer protein BRAC1, thereby preventing Keap1 from simultaneously binding to the ETGE motif of Nrf2 (Gorrini et al., 2013).

B8. Acetylation of Nrf2 by p300/CBP

It has been found that acetylation of the Neh1 domain of Nrf2 can increase the binding affinity of Nrf2-Maf heterodimers for ARE sequences. p300/CBP acetylates lysine residues of the Neh1 domain and enhances the interaction between Nrf2 and ARE sequence of antioxidant genes promoter resulting in induction of the respective genes' transcription (Sun et al., 2009).

Other mechanistic models which have been proposed for Nrf2 stabilization and activation include the oxidation-induced dissociation of the CRL^{keap1}complex, and the nucleocytoplasmic shuttling of Keap1 (Rachakonda et al., 2008; Eggler et al., 2009; Baird and Dinkova-Kostova, 2011).

Repression of Nrf2 signaling

A. Transcriptional repression of the NFE2L2 gene

A CpG island has been identified in the 5' flanking region of the NFE2L2 gene that extends to position -1175. The first 5 CpGs in this CpG island are found to be hypermethylated in prostate cancer samples and prostate cancer cell lines compared to normal prostate issues and cells. This hypermethylation leads to repression of NFE2L2 gene expression, potentially favouring tumorigenesis (Yu et al., 2010).

B. Post-transcriptional repression of Nrf2

At the post-transcriptional level, various micro RNAs (miRNAs) have been identified to interact with the Nrf2 mRNA resulting in repression of Nrf2 expression, including miR-27a, miR-28, miR-93, miR-142-5p, miR144 and miR-153 (Hayes and Dinkova-Kostova, 2014).

C. Post-translational repression of Nrf2

C1. CRLkeap1 complex-mediated degradation of Nrf2

As mentioned, the CRL^{keap1} complex is responsible for the ubiquitination and 26S degradation of Nrf2 under normal conditions. Keap1 acts as an adaptor protein to mediate the interaction between Nrf2 and the Cul3 E3-ligase enyme, resulting in ubiquitination of lysines residues of the region located between the ETGE and DLG motifs in the Neh2 domain. Thereafter, ubiquitinated Nrf2 undergoes degradation by the 26S proteosome. It has been reported that the Nedd8 molecule serves as a factor of stabilization of the CRL^{keap1} complex, and that the removal of Nedd8 by the CSN signalosome causes disruption of the complex and inhibition of Nrf2 ubiquitination. CAND1 is a mediator protein that can also block the degradation process of Nrf2 (Villeneuve et al., 2010).

C2. Crm1-dependent nuclear export and β-TrCP-dependent degradation of Nrf2

Nrf2 degradation by Keap1 is mediated by interaction via the Nrf2 Neh2 ETGE and DLG motifs. Nevertheless, in Nrf2 proteins mutant for the ETGE and DLG motifs, it has been observed that the Neh6 domain accounts for some of the residual instability of Nrf2 in a Keap1-independent way. Specifically, it has been shown that the DSGIS and DSAPGS motifs located within the Neh6 domain serve as binding sites through which Nrf2 binds with β-TrCP. β-TrCP has the ability to target Nrf2 for ubiquitination and degradation through a Skp1-Cul1-Rbx1/ Roc1 ubiquitin ligase complex; in vitro experiments with fibroblasts where β-TrCP is knocked down have shown increased Nrf2 protein levels.

This mechanism participates in the post-induction regulation of Nrf2 activity. The serine/threonine kinase GSK-3 controls the activity of the nuclear kinase Fyn which in turn phosphorylates Tyr568 of Nrf2 and promotes its Crm1 (exportin)-mediated export from the nucleus. GSK-3-mediated Fyn phosphorylation also causes an increase of the DSGIS degron activity in the Neh6 domain. The latter results in β-TrCP binding to the Neh6 domain of Nrf2 and consequently in β-TrCP-mediated Nrf2 degradation (Jain and Jaiswal, 2007; Chowdhry et al., 2013).

C3. Repression of Nrf2 by CRIF1, SIAH2 and RNF4

The CR6-interacting factor 1 (CRIF1) can promote the ubiquitination of Nrf2 through its interaction with both the N-terminal Neh2 and C-terminal Neh3 domains of Nrf2. The physiological circumstances when CRIF1 represses Nrf2 activity remain obscure.

During hypoxia, it has been observed that SIAH2 can lead to Nrf2 ubiquitination in a Neh2-independent manner. Further work is required to elucidate the basis of interaction between SIAH2 and Nrf2 and the conditions that regulate it.

It has been reported that small ubiquitin-like modifiers 1 and 2 (SUMO-1, SUMO-2) polysumoylate Nrf2 in promyelocytic leukemia

nuclear bodies.

The polysumoylated Nrf2 (pNrf2) translocates into the nucleus where SUMO-specific RING finger protein 4 (RNF4) ubiquitinates the pNrf2 leading it to degradation within the nucleus (Hayes and Dinkova-Kostova, 2014).

C4. Negative feedback loops regulating Nrf2

In vitro experiments have shown that antioxidant treatment can induce the expression of Keap1, suggesting a possible role of Nrf2 in the regulation of Keap1 expression.

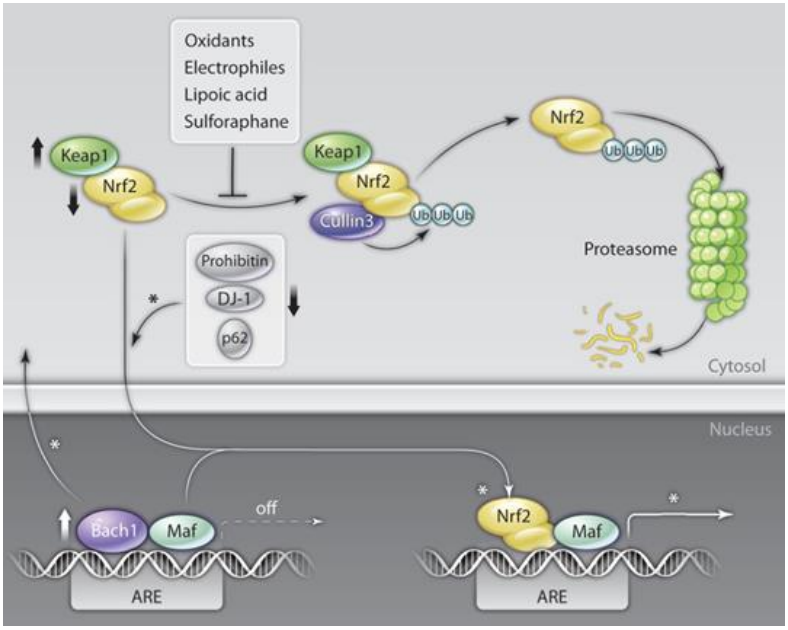
Keap1 has three ARE sequences within its promoter, of which one ARE on the reverse strand (position -46) has been demonstrated to be functional in facilitating KEAP1 gene transcription. Thus, it has been suggested that Nrf2 can control its own degradation by binding to the Keap1 ARE(-46) and thereby inducing KEAP1 transcription.

In other words, there exists an autoregulatory loop in which Nrf2 controls Keap1 at the transcriptional level and Keap1 regulates Nrf2 at the post-translational level (Lee et al., 2007).

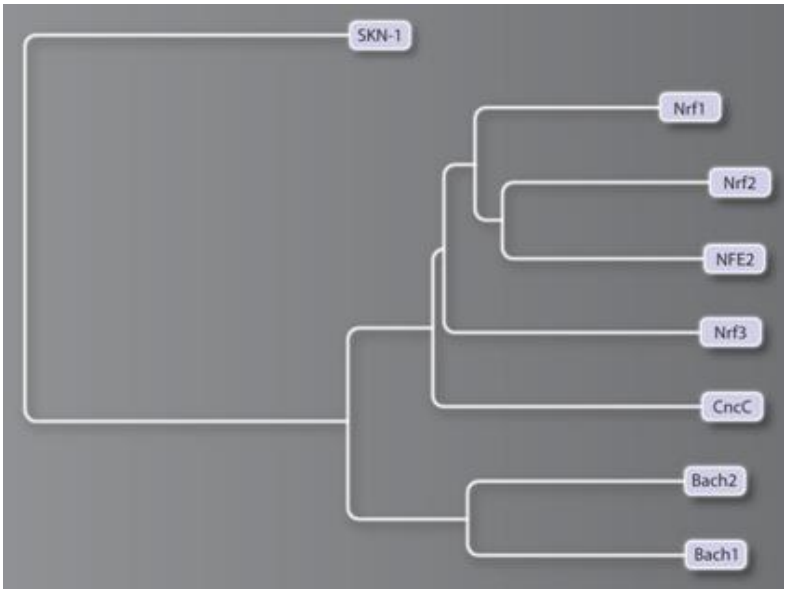
Similarly, it has been observed that Nrf2 regulates the expression of the Cul3 and Rbx1 genes.

The Cul3 and Rbx1 proteins are constituents of the CRL^{keap1} complex which is responsible for the ubiquitination of Nrf2.

Specifically, it has been found that both the Cul3 gene promoter and the Rbx1 gene promoter contain one functional ARE, and that Nrf2 acts in an autoregulatory way by binding to these AREs to regulate the expression of the Cul3 and Rbx1 genes (Kaspar and Jaiswal, 2010).



Schematic overview of the main steps in the regulation of the Keap1-Nrf2 antioxidant response pathway. Adapted from Sykiotis and Bohmann, 2010.



A phylogenetic tree of the Cnc and Bach transcription factors. A multiple species alignment was constructed with ClustalW (www.ebi.ac.uk/clustalw), and a phylogenetic tree was generated with the Jalview applet. The tree was based on the largest gap-free block of aligned sequences, which contained the DNA binding domains of the Cnc and Bach factors. Accession numbers: mouse p45 NFE2, NP_032711.2; mouse Nrf1, NP_032712.2; mouse Nrf2, NP_035032.1; mouse Nrf3, NP_035033.1; mouse Bach1, NP_031546.1; mouse Bach2, NP_001103131.1; Drosophila CncC, NP_732833.1; C. elegans SKN-1, NP_741404.1 (Sykiotis and Bohmann, 2010).

In addition, there is evidence that Nrf2 is implicated in the expression of genes encoding 26S proteasome subunits, presumably in order to increase the proteasome-dependent removal of oxidatively damaged proteins. Therefore, it has been proposed that Nrf2 may regulate this negative autoregulatory feedback loop via the proteasome to restore its levels to the basal state after the removal of oxidative stimuli (Chapple et al., 2012).

Furthermore, it is known that Bach1 competes with Nrf2 for binding to the ARE-sequence of Nrf2-regulated genes. It has been demonstrated that Bach1 transcript variant 2 has an intronic ARE sequence (position +1411) and can be a transcriptional target gene of Nrf2 (negative autoregulatory feedback mechanism) (Jyrkkänen et al., 2011).

Finally, it has been recently discovered in cancer cell lines that retinoid X receptor α (RXRα) serves as an inhibitor of Nrf2 that regulates Nrf2 activity through a direct interaction with Neh7 domain, where a RXRα-binding site has been mapped. As the activation of Nrf2 results in upregulation of RXRα, this can form another negative feedback loop for Nrf2 regulation (Wang et al., 2013).

Homology

Nrf2 belongs to a family of basic leucine zipper (bZip) transcription factors called cap'n'collar (cnc) proteins. Cnc proteins are defined by the presence of a conserved 43-amino acid cnc domain located N-terminally to the DNA-binding domain (bZip structure) and are conserved in invertebrates and vertebrates but not present in plants or fungi. The best studied cnc proteins are the C. elegans SKN-1 (Skin family member 1), the D. melanogaster Cnc (isoforms B and C), and four vertebrate counterparts: the p45 NFE2 (nuclear factor erythroid-derived 2) and the NFE2-related factors Nrf1, Nrf2 and Nrf3 ("the Nrfs"). In addition, the related transcription factors Bach1 and Bach2 are characterised by the additional presence of a BTB protein interaction domain (Sykiotis and Bohmann, 2010).

Although most Cnc factors are transcriptional activators, Bach1 and Bach2 function mainly (through not exclusively) as transcriptional repressors (figure below). Some of the Cnc proteins have important roles in development; for example, CncB is required for the development of head segments in D. melanogaster. Other family members (including Nrf2) are dispensable for development but rather contribute to the maintenance of cellular homeostasis in response to endogenous or exogenous stressors. In particular, the three Nrfs have broad and partly overlapping expression patterns and function as stress-activated transcription factors (Sykiotis and Bohmann, 2010).

Mutations

Somatic

Somatic mutations of the NFE2L2 gene have been detected in cancers. Mainly missense mutations and in frame insertions/deletions of NFE2L2 localised in the DLG and ETGE motifs of the Neh2 domain cause modifications in Nrf2 protein that lead to

impaired interaction of Nrf2 with Keap1, and thereby to constitutive Nrf2 activation.

Thus, the Nrf2 pathway is currently believed to have a role not only in cancer prevention via detoxification and maintenance of cellular homeostasis, but also in the cell growth and survival of malignant or premalignant cells. NFE2L2 mutations have been identified in oesophageal squamous cell carcinoma (8/70, 11.4%; 6/32, 18.8%), skin cancer (1/17, 6.3%; 1/22, 4.5%), non-small cell lung carcinoma (NSCLC, 6.9-10.7%), head and neck carcinoma (HN, 13-25%), cervical cancer (1/18, 5.6%) and papillary renal cell carcinoma (2 cases).

Interestingly, lung cancer, and particularly non-small cell lung cancer, has been investigated for NFE2L2 mutations in various patient populations, and in all studies the presence of NFE2L2 mutations was positively correlated with smoking history. In addition, it has been observed that the frequency of NFE2L2 gene mutations is higher in lung squamous cell carcinoma than in lung adenocarcinoma (Shibata et al., 2008a; Kim et al., 2010; Solis et al., 2010; Shibata et al., 2011; Hu et al., 2012; Gantildeán-Gómez et al., 2013; Ooi et al., 2013).

NFE2L2 polymorphisms

Specific polymorphisms associated with disease risk*

Respiratory disorders

Heterozygosity (T/G) for rs6721961 (T/C/G) has been associated with increased risk of acute lung injury (ALI) in patients with major trauma in Caucasian/African-American and Japanese populations. Paradoxically, in a Japanese cohort, the haplotype (rs2001350T/rs6726395A/ rs1962142A/rs2364722A/rs6721961T) containing the homozygous SNP rs6721961 TT has been correlated with lower annual decline in forced expiratory volume in one second (FEV1), a measure of pulmonary function. In contrast, the rs6726395 G allele showed association with higher annual decline of FEV1 induced by cigarette smoking in Japanese.

Furthermore, a haplotype containing rs35652124 C, rs6706649 C, rs6721961 G and GGC4 (a repeat polymorphism) has been proposed as a predictor factor of increased respiratory failure development in German patients with chronic obstructive pulmonary disease (COPD). A further study in a Netherlands population showed correlations between rs1806649 C and reduced COPD mortality, and between the rs2364723 CC and reduced FEV1.

In a Hungarian population of childhood asthma, rs6721961 T and rs2588882 G have been inversely correlated with the infection-induced asthma.

Cardiovasular disorders

The rs6721961 TT genotype has been associated with higher systolic and diastolic blood pressure in Japanese haemodialysis patients than the CC or CT genotypes.

Similarly, haemodialysis patients with the rs35652124 TT genotype had higher diastolic blood pressure and higher cardiovascular mortality than CC or CT carriers.

Finally, in a Netherlands population, it has been demonstrated that carriers of the rs2364723 (G/C) minor G allele showed lower triglyceride levels and reduced risk of cardiovascular mortality.

Gastrointestinal disorders

The rs6706649 C and rs35652124 C SNPs have higher frequency in Japanese patients with ulcerative colitis, and their presence has been correlated with a chronic continuous disease phenotype. In Helicobacter pylori-infected patients, the rs6706649C/rs35652124C and rs6706649C/rs35652124T haplotypes have been correlated with increased and decreased risk, respectively, of CpG methylation; rs6706649C/rs35652124T carriers with negative Helicobacter pylori test showed reduced risk of gastric cancer.

Autoimmune disorders

In a Mexican Mestizo population it was found that lupus nephritis in women was significantly associated with presence of the heterozygous rs35652124 (C/T).

Breast cancer

Homozygosity for rs6721961 (TT) or rs2706110 (TT) has been associated with increased risk of breast cancer in a Finish population. Moreover, presence of the rs6721961 T allele together with the intronic rs1962142 A allele was associated with reduced Nrf2 expression in breast cancer tissue. In a study of a Finish population, Nrf2 rs2886182 (T/C) rare homozygous genotype TT has been significantly associated with poorer survival and recurrence-free survival in patients with breast cancer that had received adjuvant chemotherapy, and with poorer survival in patients with breast cancer that had undergone postoperative radiotherapy.

Venous thromboembolism

In postmenopausal women the rs6721961 (T allele) increased the risk of venous thromboembolism after oral estrogen therapy.

Neurodegenerative diseases

In Swedish populations, a protective effect against Parkinson's disease has been detected for a haplotype containing promoter SNPs rs7557529C/ rs35652124T/ rs6706649C/ rs6721961G as well as intronic SNPs rs2886161T/ rs1806649T/ rs2001350T/ rs10183914T) (Yamamoto et al., 2004; Marzec et al., 2007; Arisawa et al., 2008; Siedlinski et al., 2009; Masuko et al., 2011; Hartikainen et al., 2012; Cho, 2013; Figarska et al., 2014; Shimoyama et al., 2014).

* The nucleotides for each SNP correspond to the map on chr. 2, and are thus complementary to the gene sequence (NFE2L2 lies on the reverse strand).

Implicated in

Various cancers

The dual role of Nrf2: NFE2L2 has been found to have both cancer chemopreventive activity (by protecting cells from carcinogen-induced damage and transformation) and oncogenic activity (by conferring a survival advantage to pre-malignant or malignant cells). Thus, on the one hand, activation of Nrf2 upregulates various conjugating enzymes for the detoxification of chemical carcinogens and protects from carcinogenicity, mutagenicity and other forms of toxicity. Experimental disruption of Nrf2 is associated with increased susceptibility of cells to carcinogens. The chemopreventive properties of Nrf2 have been demonstrated in several experimental models of cancer including colon, bladder, lung, stomach, breast, skin and liver cancer. Importantly, inducers of Nrf2 pathway are being tested in clinic trials for cancer chemoprevention.

On the other hand, in various cancers Nrf2 protein abundance and activity have been found to be increased, suggesting a role in tumour growth and survival. Gain of function somatic mutations in NFE2L2 gene which lead to disruption of the Nrf2-Keap1 binding interface complex result in upregulation of Nrf2 activity. These mutations have been identified in NSCLC, oesophageal squamous cell carcinoma, malignant melanoma, skin squamous cell carcinoma, head and neck carcinoma and cervical cancer.

In addition, it has been reported that an indirect way of upregulation of Nrf2 activity is the loss of function KEAP1 somatic mutations. These mutations have been detected in various types of cancer [lung cancer (NSCLC), thyroid papillary cancer, oesophageal cancer, gastric adenocarcinoma, hepatocellular and cholangiocellular carcinoma, gallbladder cancer, colorectal adenocarcinoma, caecum carcinoma, breast ductal carcinoma and adenocarcinoma, endometrial adenocarcinoma, ovarian serous cancer and epithelial cancer, prostate adenocarcinoma, kidney and urinary tract cancer, malignant melanoma and neuroblastoma].

Another mechanism of constitutive activation of Nrf2 in cancer cells is the silence of KEAP1 gene caused by hypermethylation of KEAP1 gene promoter. This silencing mechanism of KEAP1 gene has been detected in human lung cancer tissue cells (squamous, adenocarcinoma,
adenosquamous), lung cancer cell lines, human breast cancer tissues, colorectal cell lines, prostate cancer cell lines, human malignant gliomas and papillary thyroid cancer. Hypermethylation of CUL3 and RBX1 genes as well as CUL3, RBX1 and KEAP1 copy number losses have been proposed as further Nrf2 activation mechanisms in papillary thyroid carcinoma.

Moreover, Nrf2 expression and activation can be induced by Nrf2 cross-talk with other signalling pathways. Specifically, it has been demonstrated that in acute myeloid leukaemia (AML) Nrf2 overexpression is driven by abnormal expression of Nuclear Factor-κB (NF-κB). In addition, in NSCLC cell lines constitutive activation of mutant epidermal growth factor receptor (EGFR) and RagD-mediated activation of mammalian target of rapamycin (m-TOR) signalling pathway cause overactivation of Nrf2 as well as Nrf2-mediated resistance to EGFR-tyrosine kinase inhibitor and m-TOR inhibitor, respectively. In renal cancer cells, Nrf2 activity has been found to be increased by downregulation of E-cadherin which normally forms a quanternary complex with Nrf2, Keap1 and β-catenin and facilitates Keap1-mediated ubiquitination of Nrf2. Finally, it has been observed that transcriptional coactivator amplified in breast cancer 1 (AIB1) stimulates Nrf2 activation in cholangiocarcinoma cells inducing tumour proliferation and chemoresistance.

Thus, AIB1 has been proposed as a Nrf2 coactivator.

In summary, the upregulation of Nrf2 has antioxidant as well as cytoprotective effect in cancer cells. Especially, cytoprotective activity of Nrf2 can be exploited by cancer cells not only to face their oxidant tumour microenvironment, but also confer chemo- or/and radio- resistance during anticancer therapies. Consequently, suppression of Nrf2 activity in cancer cells inhibits tumour growth and enhances the efficacy of chemotherapeutic agents. Therefore, Nrf2 could be a target not only for cancer chemoprevention (via activating compounds) but also for cancer treatment (via inhibitors) (Shibata et al., 2008a; Shibata T. et al, 2008b; Wang et al., 2008; Chen et al., 2010; Solis et al., 2010; Yoo et al., 2010; Kim et al., 2010; Shibata et al., 2010; Wang et al., 2010; Muscarella et al., 2011; Shibata et al., 2011; Chen Q et al., 2012; Guo et al., 2012; Hanada et al., 2012; Hu et al., 2012; Kim et al., 2012; Liao et al., 2012; Sporn and Liby, 2012; Yamadori et al., 2012; Barbano et al., 2013; Ga&ntildeán-Gómez et al., 2013; Martinez et al., 2013; Shelton and Jaiswal, 2013; Shin et al., 2013; Ziros et al., 2013; Zhang et al., 2013; Funes et al., 2014; Gorrini et al., 2014; Ji et al., 2014; Onodera et al., 2014; Schultz et al., 2014).

Exogenous Nrf2 inducers		
Natural compounds	Sulphoraphane <Brassica oleracea var. italica (Brassicaceae) or broccolii & other cruciferous vegetables	Kensler T.W. et al, 2000
	Curcumine < Curcuma longa (Zingiberaceae)	Balogun E. et al, 2003
	Epigallocatechin – 3 – gallate < white tea, green tea, black tea	Shen G. et al, 2005
	Resveratrol < Veratrum album (Liliaceae)	Kode A. et al, 2008
	Caffeic acid phenethyl ester < Coffea arabica / Coffea canephora (Rubiaceae) Eucalyptus globulus (Myrtaceae) Phelinus linteus (Hymenochaetaceae)	Balogun E. et al, 2003
	Wasabi < Wasabia japonica (Brassicaceae)	Morimitsu Y. et al, 2002
	Cafestol < Coffea arabica (Rubiaceae)	Cavin C. et al, 2002
	Cinnamomyl products < Cinnamomum spp. (Lauraceae)	Liao B.C. et al, 2008
	Zerumbone < Zingiber cassumunar (Zingiberaceae)	Nakamura Y. et al, 2004
	Diallyl- di/tri sulfide < Allium sativum – garlic (Alliaceae or Liliaceae)	Gong P. et al, 2004
	Lycopene < Solanum lycopersicum – tomato plant (Solanaceae)	Ben-Dor A. et al, 2005
	Carnosol < Rosmarinus officinalis / Salvia pachyphylla (Lamiaceae)	Satoh T. et al, 2008
	Avicins < Acacia victoriae (Leguminosae)	Haridas V. et al, 2005
Synthetic compounds	Oltipraz	Ramos-Gomez M. et al, 2001
	CDDO	Liby K. et al, 2005
	CDDO-Im	Liby K. et al, 2005

Exogenous Nrf2 inducers.

Disease

Lung cancer, thyroid cancer, ovarian cancer, breast cancer, prostate cancer, endometrial cancer, cervical cancer, gastric cancer, oesophagus cancer, colorectal cancer, gallbladder cancer, liver cancer, skin cancer, acute myeloid leukemia.

Airway diseases

Oxidative stress has been associated with the pathogenesis of many acute and chronic airway disorders.

ALI and its severe form ARDS are characterised by severe systemic hypoxemia in seriously ill patients. Hypoxemia is associated with production of excessive ROS, and thus oxidative stress is a major contributor to the pathogenesis of ALI. In such hypoxic situations oxygen is one of the most commonly used supplemental therapeutic agents. However, oxygen supplementation-induced hyperoxia can also cause lung injury and airway inflammation. Nrf2 has been proposed as a hyperoxia susceptibility gene that modulates ALI in vivo.

Emphysema is characterised by loss of pulmonary elasticity as a result of permanent alveolar wall destruction and represents the alveolar lesion in COPD. Cigarette smoke is a major contributor to emphysema and COPD pathogenesis. Chronic exposure to cigarette smoke in Nrf2 knockout (KO) mice causes more severe emphysema than in wild type mice. This is associated with greater levels of inflammation, oxidative stress and endothelial and epithelial cell apoptosis in the Nrf2 KO mice.

LPS (lipo-polysaccharide)-induced septic shock in Nrf2 KO mice results in premature mortality in comparison with wild type mice, and non-lethal exposure to LPS results in greater lung inflammation and injury in Nrf2 KO mice.

Regarding asthma disease, it has been observed that the disruption of Nrf2 can cause severe airway inflammation and airway hyper-responsiveness in mouse models of asthma.

IPF is a fibroproliferative disease thought to be triggered by repeated alveolar epithelial cell injury. Rodent models of bleomycin-induced lung fibrosis have been used to study IPF. The pulmonary fibrogenic effects of bleomycin are antagonised by antioxidant enzymes like SODs in rodents. Moreover, it has been noticed that Nrf2 KO mice treated with bleomycin had elevated levels of TGF-β, the main fibrogenic factor.

Finally, it has been reported that Nrf2 has a protective role against airway infection by RSV in mice (Reddy, 2008; Cho and Kleeberger, 2010).

Disease

Acute lung injury (ALI), emphysema/chronic obstructive pulmonary disease (COPD), lung disorder during sepsis [LPS (lipo-polysaccharide)-induced septic shock], asthma/allergic airway diseases, idiopathic pulmonary fibrosis (IPF), viral airway disease (RSV - respiratory syncytial virus)

Cardiovascular disease

Nrf2 is expressed in the cardiovascular system (heart and blood vessels), and Nrf2 signaling is implicated in the regulation of vascular homeostasis and in the

prevention of cardiac hypertrophy and heart failure via suppression of oxidative stress. Although Nrf2 has been proposed as a therapeutic target in cardiovascular diseases like atherosclerosis, there is some evidence that reveal the role of Nrf2 as pro-atherogenic factor via a different mechanism. Nevertheless, modulation of Nrf2 has been supported for the prevention and the treatment of heart diseases (Sussan et al., 2008; Koenitzer and Freeman, 2010; Freigang et al., 2011).

Diabetes, diabetic nephropathy, diabetic neuropathy

Nrf2-mediated expression of endogenous cytoprotective enzymes and other antioxidant molecules has been shown to be an adaptive defence mechanism against high glucose-induced oxidative damage in diabetes. Diabetic nephropathy and neuropathy have been studied in correlation with the activity of Nrf2 pathway, and it has been found that Nrf2 exerts a protective role against these long-term complications of diabetes (Jiang et al., 2010; Cheng et al., 2011; Negi et al., 2011).

Obesity/metabolic syndrome

While it appears that the Nrf2 pathway is a regulator of energy metabolism, its precise effects and the underlying mechanisms are still controversial. In some contexts Nrf2 is protective and high-fat diet-induced obesity, while in others it is a contributing factor to metabolic disease. Nrf2 can induce several metabolic regulators in adipose tissue and liver such as PPARγ, C/EBPβ and AhR, and it can repress others such as FGF21. The exact mechanisms by which Nrf2 cross-talks with these factors are the focus of ongoing research (Chartoumpekis and Kensler, 2013).

Liver and gastrointestinal diseases

Nrf2 has been demonstrated to be a key factor dictating susceptibility to oxidative and chemical-induced injury in the gastrointestinal system. In vivo experiments have revealed that Nrf2 KO mice are more susceptible to acetaminophen-induced hepatocellular injury, benzo(a)pyrene-induced tumour formation, and Fas- and TNFα-mediated hepatocellular apoptosis. In addition, Nrf2 may be important in protecting against liver fibrosis and gallstone development. Regarding intestinal diseases, it has been suggested that Nrf2 plays an important role in the maintenance of intestinal integrity and may serve as novel target for therapies to prevent or treat Crohn's disease or ulcerative colitis (Aleksunes and Manautou, 2007).

Disease

Drug-induced hepatotoxicity, hepatocellular apoptosis, liver fibrosis, gallstone disease, bowel diseases (Crohn's disease and ulcerative colitis), chemical carcinogenesis in the gastrointestinal system

Neurodegenerative diseases

Oxidative stress is involved in the pathogenesis of a wide range of chronic neurodegenerative diseases such as AD, PD and HD.

AD is characterised by increased accumulation in the brain of neurotoxic and oxidative elements such as iron. In addition, increased oxidative damage of proteins and lipid peroxidation has been detected in the brain of AD patients. Reactive astrocytes and activated microglia contribute to the oxidative stress observed in AD brain. The expression profile of Nrf2 and ARE-regulated proteins in AD brain tissue supports the hypothesis that Nrf2 signalling may be involved in the early stages of AD.

PD, the second most common neurodegenerative disease after AD, is characterised by the preferential loss of dopaminergic neurons in the substantia nigra. Mitochondrial dysfunction and neuroinflammation in PD play a crucial role in PD pathogenesis and the subsequent oxidative stress has been suggested to be responsible for the degeneration of nigral dopaminergic neurons. Nrf2 pathway may have a neuroprotective effect on PD and its activation may be a novel therapeutic approach.

HD is a rare neurodegenerative disorder inherited in an autosomal dominant manner. Work in mouse model has shown that Nrf2 can have neuroprotective roles against HD and might be a novel treatment target for HD. Furthermore, it has been reported that Nrf2 can also potentially protect from neuronal damage in other neurological diseases such as ALS, Freidrich's ataxia, Down syndrome, multiple sclerosis, traumatic brain injury and cerebral haemorrhages (Ramsey et al., 2007; Calabrese et al., 2008; de Vries et al., 2008; Jazwa et al., 2011; Tufekci et al., 2011).

Disease

Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease(HD), Freidrich's ataxia, amyotrophic lateral sclerosis (ALS), multiple sclerosis, Down syndrome, traumatic brain injury, cerebral haemorrhage

Rheumatoid arthritis

ROS play an important role in the pathogenesis of rheumatoid arthritis (RA), and antioxidant substances and enzymes reduce cartilage damage in animal models of RA. It has been reported that a deficiency in Nrf2-mediated antioxidant defences plays a central role in pathogenesis of RA. Oxidative stress is one of the factors that contribute to RA, and Nrf2 could play an important role in alleviating its effects (Wruck et al., 2011).

Uveitis

Uveitis is an inflammatory eye disease that can cause blindness.

The main characteristic of uveitis is an inappropriate innate immune response resulting in local tissue injury.

ROS production with secretion of inflammatory cytokines and leukocytes infiltration has been documented in a model of LPS-induced uveitis.

In this model, due to an inadequate activation of Nrf2, the induction of antioxidant and anti-inflammatory responses is also incomplete. Potentiation of the antioxidant response with an Nrf2-inducing compound led to increased enzyme expression of protective enzymes, reduced cytokine expression, and decreased leukocyte adhension, suggesting Nrf2 as a potential therapeutic target in uveitis (Nagai, 2009).

Aging

A popular "hypothesis" about the causes of aging is the "free radical theory", which considers aging as the result of progressive damage to macromolecules and cellular structures caused by exposure to endogenous and exogenous pro-oxidant substances, notably free radicals. Nrf2 and its homologues in invertebrate models of aging have been shown to exert anti-aging and pro-longevity functions. Nevertheless, Nrf2 activity generally declines with age, and this decline is associated with decreased expression and/or inducibility of antioxidant genes. Overall, it is believed that, if properly fine-tuned, the Nrf2 pathway can have life span-extending effects, and can therefore be a target for promoting longevity and extending the disease-free period of life (the "health span") (Sykiotis and Bohmann, 2010).

Genes positively regulated by Nrf2 in humans (Hayes J.D. & Dinkova-Kostova A.T., 2014)		
General biochemical function	Symbol	Name
A. Detoxification: Phase I drug oxidation, reduction and hydrolysis	AKR1B1	Aldo-keto reductase family 1, member B1 (and 1B8 and 1B10)
	AKR1C1	Aldo-keto reductase family 1, member C1 (and 1C2 and 1C3)
	ALDH3A1	Aldehyde dehydrogenase 3 family, member A1 (and 3A2)
	CBR1	Carbonyl reductase 1(and 3)
	EPHX1	Epoxide hydrolase 1, microsomal
	PTGR1	Prostaglandin reductase 1 (also called LTB4DH)
	NQO1	NAD(P)H: quinone oxidoreductase 1
B. Detoxification: Phase II drug conjugation	MGST1	Microsomal glutathione S-transferase 1 (and 2)
	SULT1A1	Sulfotransferase family, cytosolic, 1A, member 1(2)
	UGT1A1	UDP glucronosyltransferase 1 family, polypeptide A1 (and 1A6)
	UGT2B7	UDP glucuronosyltransferase 2 family. Polypeptide B7 (and 2B34)
C. Detoxification: Phase III drug transport	ABCB6	ATP-binding cassette, subfamily B (MDR/TAP), member 6
	ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 1
	ABCC3	ATP-binding cassette, subfamily C (CFTR/MRP), member 3
D. Antioxidant: GSH-based system	GCLC	Glutamate-cysteine ligase, catalytic subunit
	GCLM	Glutamate-cysteine ligase, modifier subunit
	GGT1	Gamma-glutamyltransferase 1
	GLRX	Glutaredoxin 1
	GLS	Glutaminase
	GPX2	Glutathione peroxidise 2
	GSR1	Glutathione reductase
	SLC7A11	Cystine/glutamate transporter
E. Antioxidant: TXN-based system	PRDX1	Peroxiredoxin 1
	PRDX6	Peroxiredoxin 6
	SRXN1	Sulfiredoxin-1
	TXN1	Thioredoxin
	TXNRD1	Thioredoxin reductase 1
F. Carboxylate metabolism and NADPH regeneration	HDK1	Hexokinase domain containing 1
	ME1	Malic enzyme 1, NADP+-dependent, cytosolic
	PGD	6-phosphogluconate dehydrogonase
	TALDO1	Transaldolase
	TKT	Transketolase isoform 1
	UGDH	UDP-glucose dehydrogonase
G. Heme and iron metabolism	BLVRA	Billiverdin reductase A
	BLVRB	Billiverdin reductase B (flavin reductase (NADDPH))
	FECH	Ferrochelatase
	FTH1	Ferritin, heavy polypeptide 1
	FTHL12	Ferritin, heavy polypeptide-like 12
	FTHL17	Ferritin, heavy polypeptide-like 17
	FTL1	Ferritin, light polypeptide
	HMOX1	Heme oxygenase (decycling) 1
H. Transcription factors and associated proteins	MAFG	MafG protein
	NFE2L2	Nuclear factor-erythroid 2-like 2
	PPARG	Peroxisome proliferator-activated receptor gamma (PPARγ)
	PPRAGC1B	Peroxisome proliferator-activated receptor gamma coactivator 1-beta
	RXRA	Retinoid X receptor alpha (RXRα, or NR2B1)
I. Ubiquitin ligase substrate adaptor	KEAP1	Kelch-like ECH-associated protein 1

Genes positively regulated by Nrf2 in humans.

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