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KEAP1/NRF2 as a druggable target

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Abstract

Nuclear factor erythroid 2-related factor 2 (NRF2; encoded by *NFE2L2*) is an inducible transcription factor that regulates the expression of a large network of genes encoding proteins with cytoprotective functions. NRF2 also has a role in the maintenance of mitochondrial and protein homeostasis, and its activation allows adaptation to numerous types of cellular stress. NRF2 is principally regulated at the protein stability level by three main ubiquitin ligase systems, of which the regulation by Kelch-like ECH-associated protein 1 (KEAP1), a substrate adaptor protein for Cul3/Rbx1-based ubiquitin ligase, is best understood. KEAP1 is a multi-functional protein and, in addition to being a substrate adaptor, it is a sensor for electrophiles and oxidants. Pharmacological inactivation of KEAP1 has protective effects in animal models of human disease, and KEAP1 is now widely recognized as a drug target, particularly for chronic diseases, where oxidative stress and inflammation underlie pathogenesis. Many compounds that target KEAP1 have been developed, including electrophiles that bind covalently to cysteine sensors in KEAP1, non-electrophilic protein-protein interaction inhibitors that bind to the Kelch domain of KEAP1, disrupting its interaction with NRF2, and most recently, heterobifunctional proteolysis-targeting chimeras (PROTACs) that promote the proteasomal degradation of KEAP1. The drug development of KEAP1-targeting compounds has led to the entry of two compounds, dimethyl fumarate (BG-12, Tecfidera®) and RTA-408 (omaveloxolone, SKYCLARYS®), in clinical practice. In 2013, dimethyl fumarate was licenced as the first oral first-line therapy for relapsing-remitting multiple sclerosis and is also used for the treatment of moderate-to-severe plaque psoriasis. In February 2023, omaveloxolone was approved by the United States Food and Drug Administration as the first and only drug for patients with Friedreich's ataxia.

Key words: Electrophile, KEAP1, NQO1, NRF2, omaveloxolone, sulforaphane

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NRF2

Nuclear factor erythroid 2-related factor 2 (NRF2; encoded by the *NFE2L2* gene) is a member of the cap'n'collar (CNC) basic-region leucine zipper transcription factor family (1). NRF2 is comprised of seven domains termed NRF2-ECH homology (Neh) 1–7 domains (Figure 1A). In the nucleus, NRF2 forms a heterodimer with members of the small musculoaponeurotic fibrosarcoma (sMAF) family of transcription factors, and the heterodimer controls the expression of approximately 250 genes, which contain antioxidant/electrophile response elements (AREs) in their regulatory regions (2). Following translation, the resulting proteins have widely cytoprotective functions, including drug-metabolizing, antioxidant, and anti-inflammatory, and also have roles in the maintenance of mitochondrial and protein homeostasis (1, 3).

Regulation of NRF2

Under homeostatic conditions, NRF2 is a short-lived unstable protein, with a half-life of just a few minutes. Three main ubiquitin ligase systems regulate the protein stability of NRF2: Kelch-like ECH-associated protein 1 (KEAP1), a substrate adaptor protein for Cul3/Rbx1-based ubiquitin ligase; β-TrCP, a substrate adaptor for Skp1-Cul1/Rbx1-based ubiquitin ligase; and the endoplasmic reticulum (ER)-residing HRD1 (Figure 1B). Following ubiquitination, NRF2 undergoes rapid proteasomal degradation (1).

KEAP1 is the main negative regulator of NRF2 (4). NRF2 binds to the Kelch domain of KEAP1 (which forms a β-propeller structure) via the N-terminal Neh2 domain of the transcription factor, which has a low-affinity DLGex motif (forming a three-helix structure) and a high-affinity ETGE motif (forming a β-hairpin structure) (5, 6). This two-site binding of NRF2 to the KEAP1 homodimer is an essential requirement for the subsequent ubiquitination and proteasomal degradation of NRF2 (7). Time-lapse microscopy experiments have illustrated the degradation of NRF2 by KEAP1 in single live cells co-expressing fusion proteins of EGFP-KEAP1 and NRF2-mCherry (8). Furthermore, experiments in cells co-expressing fusion proteins of EGFP-NRF2 and KEAP1-mCherry utilizing quantitative Förster resonance energy transfer-based multiphoton fluorescence lifetime imaging microscopy (FRET-FLIM) revealed that KEAP1 uses a cyclical mechanism to target NRF2 for degradation (9-11). According to this mechanism, the interaction between KEAP1 and NRF2 follows a cycle whereby the KEAP1/NRF2 protein complex sequentially adopts two distinct conformations, "open," in which NRF2 interacts with a single molecule of KEAP1, followed by "closed," in which NRF2 binds to both members of the KEAP1 dimer; in this conformation, NRF2 undergoes ubiquitination and subsequent proteasomal degradation, and free KEAP1 is regenerated.

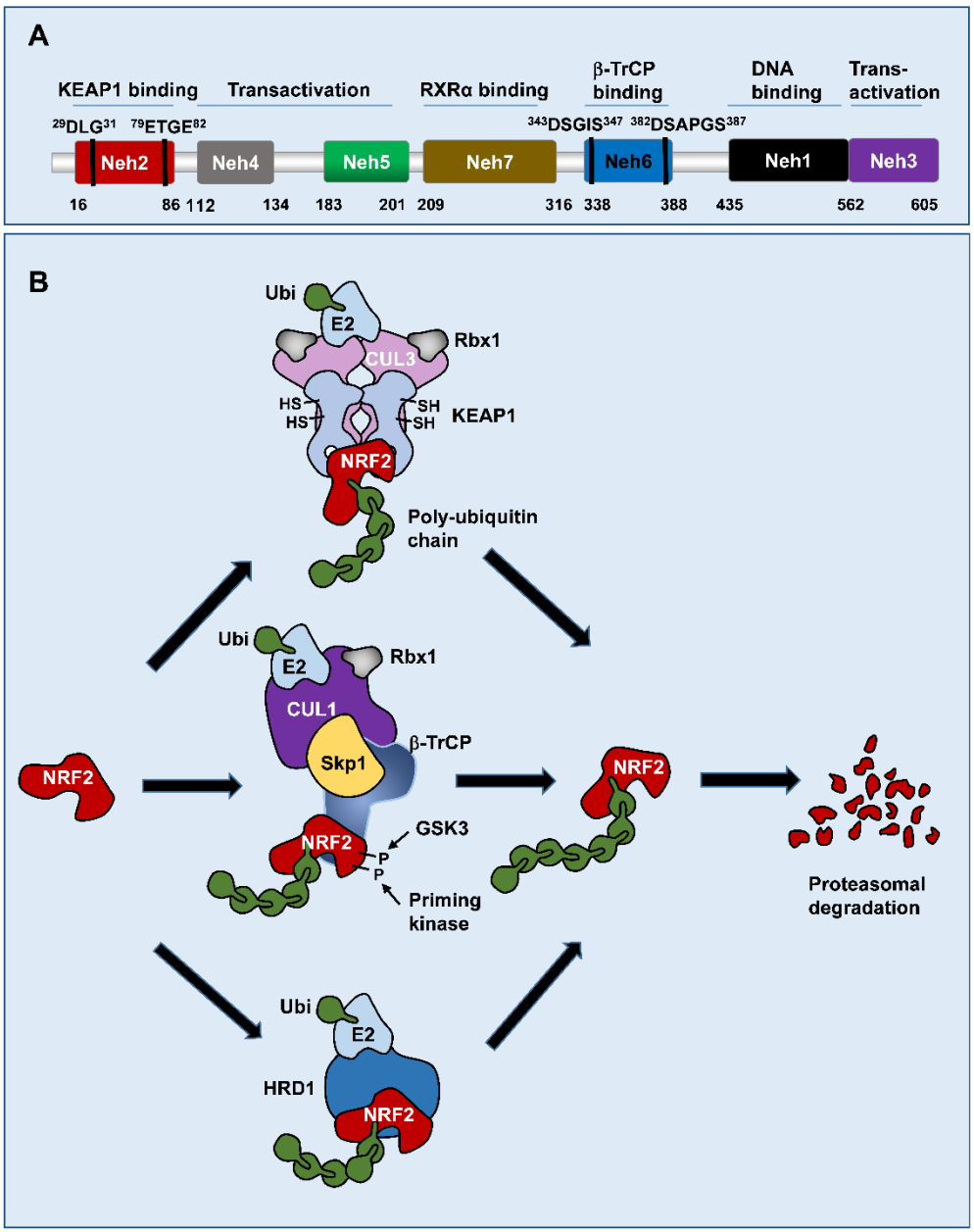

Figure 1. **(A) Domain structure of NRF2. NRF2 binds KEAP1 through its N-terminal Neh2 domain. Neh4 and Neh5 are transactivation domains through which NRF2 recruits cAMP response element-binding protein (CREB)-binding protein (CBP) and/or receptor-associated coactivator 3 (RAC3). Through its Neh7 domain, NRF2 binds to retinoid X receptor alpha (RXRα), one of the negative regulators of NRF2. Through its Neh6 domain, NRF2 binds to β-transducin repeat-containing protein (β-TrCP). NRF2 binds to sMAF and DNA through its Neh1 domain. The C terminal Neh3 domain of NRF2 recruits chromo-ATPase/helicase DNA-binding protein 6 (CHD6). Also shown are the low affinity binding 'DLG' motif and the high affinity binding**

'ETGE' motif in the N-terminal Neh2 domain, through which NRF2 binds to KEAP1, as well as the phosphodegron in the Neh6 domain through which, following phosphorylation by glycogen synthase kinase 3 (GSK3), NRF2 binds to β-transducin repeat-containing protein (β-TrCP). (B) Regulation of NRF2. NRF2 is regulated primarily at the protein stability level by three main ubiquitin ligase systems: KEAP1, a substrate adaptor protein for Cul3/Rbx1-based ubiquitin ligase; β-TrCP, a substrate adaptor for Skp1-Cul1/Rbx1-based ubiquitin ligase; and the endoplasmic reticulum (ER)-residing HRD1. KEAP1-mediated degradation of NRF2 requires that the cysteine sensors of KEAP1 are in the reduced state. To bind β-TrCP, NRF2 has to be phosphorylated by glycogen synthase kinase 3 (GSK3), which in turn requires prior phosphorylation of NRF2 by a priming kinase. NRF2 is degraded by HRD1 during ER stress. The ubiquitinated NRF2 is then targeted for proteasomal degradation.

Slika 1. **(A) Struktura domena NRF2. NRF2 vezuje KEAP1 preko svog N-terminal Neh2 domena. Neh4 i Neh5 su transaktivacioni domeni putem kojih NRF2 angažuje CREB-vezujući protein (CBP) ili koaktivator 3 povezan sa receptorom (RAC3). Putem svog Neh7 domena, NRF2 se vezuje za retinoid X receptor alfa (RXRα), jedan od negativnih regulatora NRF2. Putem svog Neh6 domena, NRF2 se vezuje za protein koji sadrži ponovke β-transducina (β-TrCP). NRF2 se vezuje za sMAF i DNK putem svog Neh1 domena. C-terminalni Neh3 domen NRF2 angažuje CHD6. Takođe su prikazani i vezni motiv niskog afiniteta 'DLG' i vezni motiv visokog afiniteta 'ETGE' u N-terminalnom Neh2 domenu, kojima se NRF2 vezuje za KEAP1, kao i fosfodegron u Neh6 domenu kojim se, nakon fosforilacije glikogen-sintaza-kinazom 3 (GSK3), NRF2 vezuje za protein koji sadrži ponovke β-transducina (β-TrCP). (B) Regulacija NRF2. NRF2 primarno regulišu tri glavna sistema ubikvitin ligaze: KEAP1, supstratni adapter za Cul3/Rbx1-zavisnu ubikvitin ligazu; β-TrCP, supstratni adapter za Skp1-Cul1/Rbx1-zavisnu ubikvitin ligazu; i HRD1 koji se nalazi u endoplazmatičnom retikulumu (ER). KEAP1-posredovana razgradnja NRF2 zahteva da cisteinski senzori KEAP1 budu u redukovanom stanju. Kako bi vezao β-TrCP, NRF2 mora da bude fosforilovan glikogen-sintaza-kinazom 3 (GSK3), što zahteva prethodnu fosforilaciju NRF2 prajming kinazom. HRD1 razlaže NRF2 tokom ER stresa. Ubikvitinovani NRF2 potom postaje meta za proteazomsku razgradnju.**

Pharmacological targeting of KEAP1

KEAP1 is a multi-functional protein and, in addition to its substrate adaptor function, it also serves as a sensor for exogenous and endogenous electrophiles and oxidants (12-14). This function is due to the fact that KEAP1 is equipped with highly reactive cysteine residues which, when chemically modified, such as via oxidation or Michael addition reactions, disrupt the cycle of KEAP1-mediated degradation of NRF2 (9). This leads to accumulation of the KEAP1/NRF2 protein complex in the "closed

conformation", without release of NRF2 (9, 10, 15). As a result, free KEAP1 is not regenerated, newly-synthesized NRF2 is stabilized, and following nuclear translocation, activates transcription of its target genes. Some examples of electrophilic NRF2 activators are given in Figure 2.

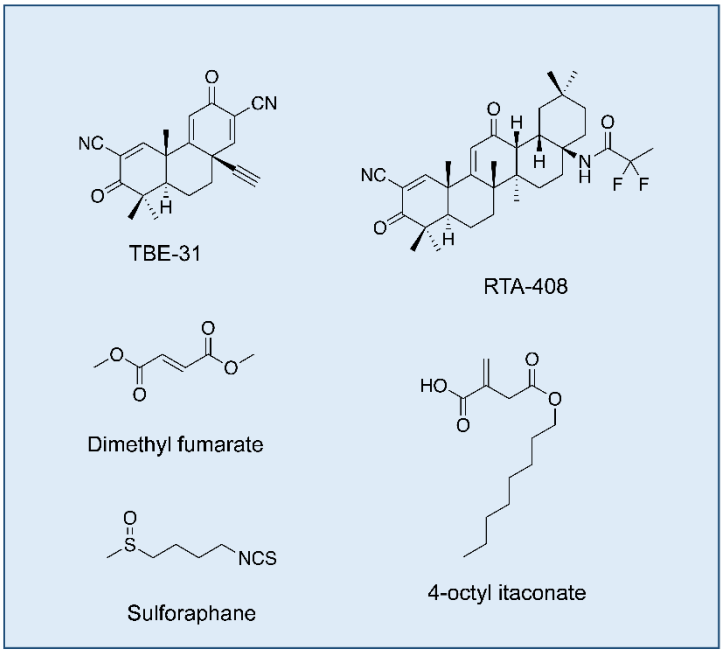

Figure 2. Examples of electrophilic NRF2 activators
Slika 2. Primeri elektrofilnih NRF2 aktivatora

KEAP1 is also a target for non-electrophilic small molecules, all of which are designed to bind to the Kelch domain of the protein. In contrast to electrophiles, binding of these compounds to KEAP1 inhibits its interaction with NRF2; such compounds are known as KEAP1-NRF2 protein-protein interaction (PPI) inhibitors. Experiments using titration nuclear magnetic resonance (NMR) spectroscopy have revealed that, in contrast to electrophiles, which do not cause dissociation of NRF2, non-electrophilic PPI inhibitors conform to the "hinge-and-latch" mechanism of NRF2 activation, whereby they cause dissociation of the weaker KEAP1-DLGex interaction (as a latch), leaving intact the strong KEAP1-ETGE interaction (as a hinge) (15).

Based on the crystal structure of the Kelch domain of KEAP1, several peptide and small-molecule PPI inhibitors have been designed and tested as NRF2 activators (16). Some examples of non-electrophilic small-molecule NRF2 activators are given in Figure 3. Interestingly, some PPI inhibitors bearing a phenyl bis-sulfonamide moiety, such as compound 11 (Figure 3), bind to KEAP1 in a "peptidomimetic" conformation, resembling the KEAP1-Kelch : NRF2-ETGE peptide complex (17). A structure-based virtual screen of more than one billion compounds identified small-molecule PPI

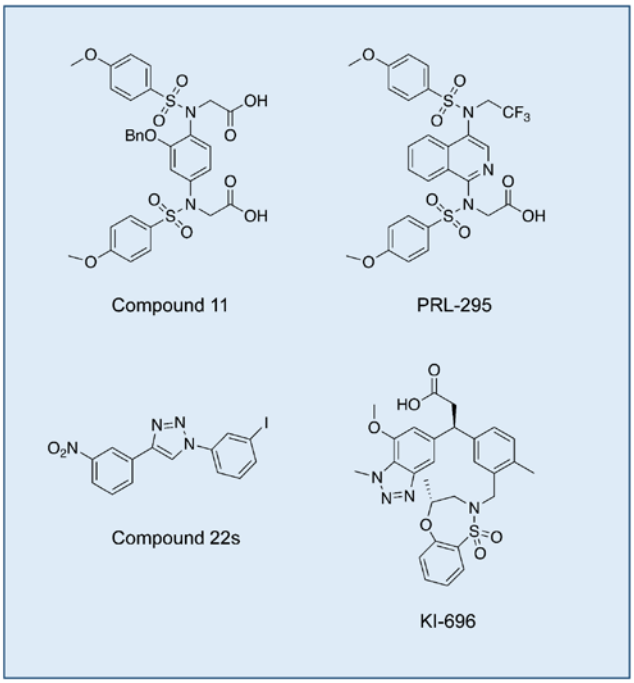

Figure 3. Examples of non-electrophilic NRF2 activators which bind to the Kelch domain of KEAP1, inhibiting the KEAP1-NRF2 protein-protein interactions. These compounds are also known as protein-protein interaction (PPI) inhibitors.

Slika 3. Primeri neelektrofilnih NRF2 aktivatora koji se vezuju za Kelch domen KEAP1, inhibirajući KEAP1-NRF2 protein-protein interakcije. Ova jedinjenja su poznata i kao inhibitori protein-protein interakcija (PPI).

inhibitors with nanomolar affinities for KEAP1 *in vitro* (18). Nonetheless, most non-electrophilic PPI inhibitors are less potent in activating NRF2 in cell-based systems in comparison with the most potent electrophiles, suggesting that they are not taken up by cells as efficiently as the electrophiles. However, efforts to improve cellular uptake and metabolic stability have led to the design of compounds with potencies similar to electrophiles. One example is the metabolically stable isoquinoline PRL-295 (Figure 3) (19). PRL-295 has a very similar potency to the electrophile sulforaphane (Figure 2) in mouse and human cells, although the bioavailability of orally-administered PRL-295 and its ability to activate NRF2 is seen mainly in the liver (20). The ability of PRL-295 to engage KEAP1 in cells and *in vivo* was demonstrated by the use of the cellular thermal shift assay (CETSA), which showed increased thermostability of ectopically expressed fluorescently tagged KEAP1 (KEAP1-mCherry), as well as endogenous KEAP1 from cells and murine livers following treatment with PRL-295. FRET-FLIM imaging experiments of live cells co-expressing sfGFP-NRF2 and KEAP1-mCherry fusion proteins showed that treatment with this isoquinoline prolonged the fluorescence lifetime of the FRET pair donor, sfGFP-NRF2, indicating disruption of the KEAP1-NRF2 protein

complex (20). Similar experiments were also conducted with compound 22s (Figure 3), a PPI inhibitor from the 1,4-diaryl-1,2,3-triazole chemical class, further confirming the ability of PPI inhibitors to disrupt the KEAP1-NRF2 protein complex in live cells (21).

Recently, two independent groups (22, 23) have employed targeted protein degradation through proteolysis-targeting chimeras (PROTACs) as a strategy to design heterobifunctional degraders of KEAP1. In both cases, these KEAP1-targeting PROTACs were based on the small molecule KI-696 (Figure 3), which was originally designed to bind with high affinity to the Kelch domain of KEAP1 and potently inhibit the protein-protein interactions between KEAP1 and NRF2, leading to NRF2 activation (24). To create these PROTACs, KI-696 was linked to a ligand that binds the Cullin4-Rbx1 ligase-cereblon (CRBN) complex, resulting in the CRBN-dependent ubiquitination and subsequent degradation of KEAP1. A very important feature of this strategy, which is in contrast with classical small molecule inhibitors, is that, following the degradation of the protein of interest, the PROTAC is recycled to target another molecule of the protein of interest, making this mechanism of action catalytic and thus highly efficient. Two examples of KEAP1-targeting PROTACs, namely DGY-06-177-pk2 and compound 14, are shown in Figure 4.

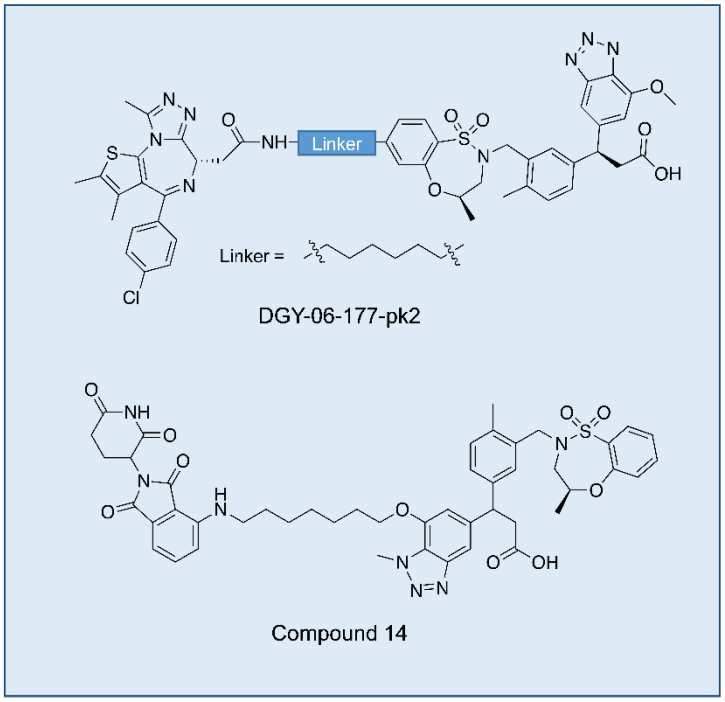

Figure 4. Examples of KEAP1-targeting PROTACs that activate NRF2 by promoting the proteasomal degradation of KEAP1

Slika 4. Primeri himera usmerenih na proteolizu (PROTACs) koje targetiraju KEAP1 što aktivira NRF2 podsticanjem proteazomske degradacije KEAP1

The cytoprotective role of NRF2

Targeting of KEAP1 by the compounds described above, regardless of the precise mechanism of action, leads to NRF2 activation and enhanced broad cytoprotection. The importance of NRF2 activation in human health has been demonstrated by the associations between functional genetic variations of *NFE2L2* and disease risk in humans. Thus, *NFE2L2* polymorphisms (*NFE2L2* is the gene that encodes NRF2), which result in lower NRF2 expression, increase the risk for diabetes mellitus (DM) (25), coronary heart disease (26, 27), age-dependent increase in vascular stiffness (28), respiratory failure in chronic obstructive pulmonary disease (COPD) patients (29), smoking-induced emphysema (30), gastrointestinal inflammation infection (31), and tuberculosis (32). Conversely, a protective haplotype allele has been identified and shown to associate with delayed onset of Parkinson's disease (PD) in a Swedish cohort and a decreased risk in a Polish cohort (33), as well as in four other independent European case-control studies (34), although not in a Taiwanese cohort (35), whereas polymorphisms leading to decreased *NFE2L2* expression occur at high frequency in a Chinese cohort of PD patients (36). Moreover, NRF2 signalling is impaired in many neurological conditions, such as Friedreich's ataxia (FRDA) (37), Huntington disease (HD) (38), and autism spectrum disorder (ASD) (39), whereas its pharmacological activation is beneficial (40).

Interestingly, in mouse neurons, epigenetic inactivation of the promoter of *Nfe2l2* leads to its repression (41), strongly suggesting that the benefits of NRF2 activation in the brain are mediated by its effects in astrocytes and microglia, the brain-resident macrophages. Notably, although the prevailing view is that NRF2 activation is protective mainly against chronic disease (16), there is also emerging evidence for a potential protective role of NRF2 against viral infections, including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) (42-44). Thus, NRF2-mediated gene expression is suppressed in lung biopsies obtained from COVID-19 patients (43), whereas the NRF2 activators 4-octyl-itaconate, dimethyl fumarate, sulforaphane (Figure 2), bardoxolone and bardoxolone methyl, inhibit SARS-CoV2 replication and/or viral infection (43, 45, 46).

The cytoprotective role of electrophilic NRF2 activators

A large body of laboratory experiments in cell culture and animal models, many of which compare wild-type and NRF2-knockout mice, has generated convincing experimental evidence that pharmacological NRF2 activation is protective against non-neoplastic disease, particularly in cases where oxidative stress and inflammation underlie the pathogenesis of the disease (16, 47).

Cyclic cyanoenones

The electrophilic cyclic cyanoenones represent some of the most NRF2 activators known to date and activate NRF2 by modifying C151 in KEAP1 irrespective of their size and shape (48-50). Thus, the acetylenic tricyclic bis(cyanoenone) TBE-31 (Figure 2) induces the classical NRF2 transcriptional target NAD(P)H: quinone oxidoreductase 1 (NQO1) in Hepa1c1c7 cells at low nanomolar concentrations, with a Concentration that

Doubles the NQO1 enzyme activity (CD value) of 1 nM (51). TBE-31 is also highly potent and bioavailable following oral administration to mice (52) and is suitable for chronic administration (53). Notably, pharmacokinetic and pharmacodynamic studies in mice have shown that, although TBE-31 has a terminal elimination half-life of 10.2 h after a single dose, its cytoprotective effects are evident for much longer periods of time, beyond the half-life of the compound (54). This is because the cytoprotection is not due to TBE-31 itself, not even due to NRF2, but due to the function of the transcriptional targets of NRF2, which are proteins with long half-lives. This long-lasting pharmacodynamic effect allows for chronic dosing with a low frequency. Thus, chronic (~30 weeks) topical application of TBE-31 twice a week to the murine skin resulted reduction in tumor multiplicity and burden in a model of cutaneous squamous cell carcinoma caused by chronic exposure to low doses of solar-simulated ultraviolet radiation (54). Oral administration of TBE-31, three times per week for three weeks, abolished the development of pre-neoplastic foci in a rat model of aflatoxin-mediated hepatocellular carcinoma (51). Similarly, oral administration of TBE-31, three times per week, decreased hepatic steatosis and fibrosis in wild-type mice fed a high-fructose plus high-fat diet, but not in their NRF2-deficient counterparts (53). In a rat model of epilepsy, administration of the closely related pentacyclic cyanoenone RTA-408 (Figure 2) during the first week after seizure onset increased the levels of ATP, prevented neuronal death in the hippocampus, and dramatically reduced (by 94%) the frequency of late spontaneous seizures for at least 4 months following status epilepticus (50).

Dimethyl fumarate

Dimethyl fumarate (DMF) (Figure 2) is one of the earliest NRF2 activators discovered. DMF was one of the compounds within a panel of fumaric acid derivatives which was found to induce the NRF2 transcriptional targets NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione *S*-transferases (GSTs) in multiple organs following oral administration to mice (55). Induction of NQO1 was also observed following DMF exposure of human peripheral blood mononuclear cells (PBMCs) *ex vivo* and in PBMCs isolated from DMF-treated multiple sclerosis patients (56). DMF activates NRF2 by biding to C151 in KEAP1 (57-59). The same cysteine in KEAP1 is also the target of 4-octyl itaconate (Figure 2), a cell-permeable analogue of itaconate, an endogenous anti-inflammatory metabolite which forms during macrophage activation (60).

Sulforaphane

The isothiocyanate sulforaphane (Figure 2) is one of the most potent naturally occurring NRF2 activators known. It was isolated from extracts of broccoli (*Brassica oleracea* var *italica*) as the principal NQO1 inducer (61). Broccoli hybrids with high content of the sulforaphane precursor, glucoraphanin, have been developed through genome introgression from the wild species *Brassica villosa*, and commercialised as Beneforté broccoli (62, 63). Sulforaphane is now considered a classical NRF2 activator and has consistently shown induction of NRF2-transcriptional targets, including NQO1,

and demonstrated protective effects in animal models (61, 64). Induction of NQO1 was also observed in human peripheral blood mononuclear cells (PBMCs) *ex vivo* following sulforaphane exposure, as well as in PBMCs and skin biopsies isolated from human subjects that had received 3-day-old broccoli sprout extracts as a source of sulforaphan (65, 66). Sulforaphane activates NRF2 by modifying C151 in KEAP1 (57, 67, 68).

Extracts of broccoli and 3-day-old broccoli sprouts represent sources of sulforaphane and many clinical trials have used such preparations to test for their effects in healthy or at-risk subjects, including populations exposed to environmental pollutants, as well as in people with chronic low-grade inflammation, allergy, asthma, COPD, ASD, depression, schizophrenia, diabetes, metabolic syndrome, chronic kidney disease, cystic fibrosis, skin disease (such as epidermolysis bullosa simplex and pachyonychia congenita), age-associated skin ageing and cardiovascular dysfunction, prostate, breast, skin, lung and head and neck cancer (64). Sulforaphane is a highly reactive, unstable compound. Stabilized sulforaphane preparations have been developed to overcome this limitation, such as Prostaphane® and Sulforadex® (SFX-01). Prostaphane® has been used in clinical trials in patients with prostate cancer. Sulforadex® (SFX-01) has been used in patients with subarachnoid haemorrhage.

As mentioned above, the cytoprotective action of pharmacological NRF2 activators is carried out by the transcriptional targets of NRF2, which are proteins with long half-lives, allowing low frequency dosing regimes in animals. However, what dosing regime will be most beneficial in humans is still unclear, and the decision is likely to depend on the pharmacological agent as well as the specific disease. In this context, it is noteworthy that administration of an intermittent (once a week) high-dose (500 mg) of the NRF2 activator oltipraz inhibited the aflatoxin bioactivation, whereas administration of a sustained (once daily) low-dose (125 mg) of oltipraz increased the aflatoxin metabolism through the mercapturic acid pathway (69). This suggests that intermittent dosing might be more appropriate during concurrent therapy to avoid potential interference with the efficacy of the therapeutic agent, whereas sustained dosing might be more appropriate where the continuous removal of a toxic agent (e.g., ROS) is essential, such as in certain genetic diseases. If a disease is caused by a vicious cycle of inflammation causing oxidative stress leading to further inflammation and ultimately cell death, breaking that vicious cycle early in the disease pathogenesis might lead to long-term efficacy, and may allow dosing with a very low (e.g. once a month) frequency (50).

Presently, Clinicaltrials.gov shows approximately 100 clinical trials with NRF2 activators, which include pure compounds, complex plant extracts, and dietary supplements. The pentacyclic cyanoenones are particularly promising due to their high potency and efficacy (47). Indeed, several large clinical trials with bardoxolone methyl have been conducted. A Phase 3 randomized clinical trial in patients with advanced renal disease (stage 4 chronic kidney disease and type 2 diabetes mellitus) was terminated early because of observed increase in heart failure in the bardoxolone methyl group, which was associated with fluid retention (70). A subsequent study identified elevated baseline B-type natriuretic peptide and previous hospitalization for heart failure as predictors of heart

failure (71). Exclusion of patients with these risk factors has allowed the design of a new Phase 3 clinical trial with bardoxolone methyl in patients with Alport syndrome (72); this study is currently active. Moreover, two NRF2 activators, DMF (BG-12, Tecfidera®) and RTA-408 (omaveloxolone, SKYCLARYS®), are now in clinical practice. In 2013, DMF was licenced as the first oral first-line therapy for relapsing-remitting multiple sclerosis (73). DMF is also used for the treatment of moderate-to-severe plaque psoriasis (74). In February 2023, RTA-408 was licenced as the first and only drug for patients with Friedreich's ataxia based on clinical trials addressing the safety and efficacy data of this compound (75-77).

NRF2 in cancer

Given its broadly cytoprotective role, it is not surprising that NRF2 is often hyperactive in cancer and is a significant contributor to the hallmarks of cancer (78), including redox (79) and metabolic (80) adaptation, as well as resistance to chemo-, radio- and immunotherapy (81). Notably, the hyperactivation of NRF2 in cancer cells, together with transcription factor CCAAT Enhancer Binding Protein Beta (CEBPB), generates enhancers at gene loci that are not regulated by transiently activated NRF2 under physiological conditions (82). The activation of NRF2 is primarily used by cancer cells to combat the oxidative and metabolic stress that they would otherwise experience within their unfavourable growth environment. However, the persistent NRF2 activation causes metabolic imbalances, particularly within the pentose phosphate pathway and the cysteine and glutamate pools. This understanding has led to the proposal of strategies for exploiting these metabolic imbalances, including the use of small-molecule inhibitors of glutaminase (GLS) (83) or glucose 6-phosphate dehydrogenase (G6PD) (84). Currently, a Phase 1 clinical trial with the glutaminase inhibitor CB-839 is being conducted in advanced non-small-cell lung cancer patients, with a focus on those harbouring *NFE2L2* or *KEAP1* mutations (85).

The NRF2 hyperactivation in cancer has sparked an interest in developing NRF2 inhibitors for cancer treatment and overcoming resistance to therapies. Multiple approaches have been used, including chemical library high-throughput screening (HTS), fragment-based nuclear magnetic resonance spectroscopy (NMR) screening, PROTACs, and molecular glues (86). Very recently, a chimeric molecule combining a CRBN ligand with an NRF2-binding portion was synthesized and shown to induce the degradation of the NRF2-MAFG heterodimer through the proteasome (87). In short, although there are no NRF2 inhibitors in clinical trials to date, some promising compounds are beginning to emerge.

Pros and Cons of NRF2 activation as an adjunctive therapy

Including NRF2 activators together with other pharmacological agents may have positive or negative effects. For example, NRF2 activation, by increasing the levels of drug-metabolizing enzymes, may alter the pharmacokinetics of concurrent therapies. This largely depends on the function of the NRF2 transcriptional targets. Thus, genetic NRF2

activation in mice (by hepatocyte-specific disruption of the *Keap1* gene) leads to nuclear accumulation of NRF2, increased expression of multiple drug-metabolizing, including NQO1, GCLC, GPX2, and several members of the GST family, and resistance to the hepatotoxicity of acetaminophen (88). Similarly, pharmacological activation of NRF2 is protective against acetaminophen toxicity regardless of the mechanism of activation of NRF2. For example, this has been shown for withaferin A that activates NRF2 in a KEAP1-independent, phosphatase and tensin homolog (PTEN)-dependent manner (89), the C151 KEAP1-targeting electrophiles sulforaphane (90), dimethyl fumarate (91), and CDDO-Im (92), as well as the non-electrophilic KEAP1-NRF2 PPI inhibitor PRL-295 (20). Pharmacological activation of NRF2 is also protective against the phototoxicity and photocarcinogenicity of azathioprine, a widely used anti-inflammatory and immunosuppressive agent, by accelerating the excretion of thio-dGTP, the ultimate metabolite of the azathioprine pro-drug (54, 93). However, whether NRF2 activation alters the therapeutic efficacy of these drugs remains to be established.

The NRF2 transcriptional target NQO1 participates in the bioactivation of quinone-containing anticancer drugs (94). Additionally, NQO1 catalyzes the obligatory 2-electron reduction of the quinone-containing HSP90 inhibitors forming hydroquinone metabolites, which are more potent HSP90 inhibitors than their quinone-containing parent compounds; this leads to increased cytotoxicity, but also limits this increased cytotoxicity to cancer cells with hyper-active NRF2/high NQO1 levels (95). In other cases, the ultimate outcome may depend not only on the NQO1 levels, but also on the overall reductive power of the cell. One example is β-lapachone, which is bioactivated by NQO1 and thus is expected to target cells with high NQO1 levels. However, because its mechanism of action ultimately depends on the generation of reactive oxygen species, which are efficiently scavenged in cells with high NRF2 activity, such cells are in fact more resistant and not more sensitive to the toxicity of β-lapachone, despite their high NQO1 levels (96).

Notably, some of the existing therapies have been suggested to exert their beneficial effects, at least in part, via NRF2. These include the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) reductase inhibitors statins, which have antioxidant and anti-inflammatory functions that are independent of their lipid-lowering effects, but dependent on NRF2 activation (97, 98). Another example is the aromatase inhibitor exemestane (99). Interestingly, a recent study has suggested that NRF2 activation and the consequent induction of growth/differentiation factor 15 (GDF15) is responsible for some of the tissue-protective effects of certain cyclooxygenase (COX) inhibitors, such as the commonly used nonsteroidal anti-inflammatory drug (NSAID) indomethacin (100).

Concluding remarks

The extensive efforts of numerous investigators over the past three decades since the discovery of NRF2 and KEAP1 have provided an in-depth knowledge of the function and regulation of this essential cytoprotective system. A number of challenges still

remain, including how to achieve specificity, how to monitor in humans target engagement, pharmacodynamic responses and safety, which are the most appropriate disease indications (101). These will undoubtedly be the subjects of future investigations. Nonetheless, it is extremely gratifying to witness the highly significant advances in drug development of NRF2 activators, culminating in the approval of DMF and RTA-408 for clinical use.

References

1. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. Physiol Rev. 2018;98:1169-1203.
2. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313-22.
3. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci. 2014;39:199-218.
4. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13:76-86.
5. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell. 2006;21:689-700.
6. Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M. Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol Cell Biol. 2014:34;832-46.
7. McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. J Biol Chem. 2006;281:24756-68.
8. Baird L, Dinkova-Kostova AT. Diffusion dynamics of the Keap1-Cullin3 interaction in single live cells. Biochem Biophys Res Commun. 2013;433:58-65.
9. Baird L, Lleres D, Swift S, Dinkova-Kostova AT. Regulatory flexibility in the Nrf2-mediated stress response is conferred by conformational cycling of the Keap1-Nrf2 protein complex. Proc Natl Acad Sci U S A. 2013;110:15259-64.
10. Baird L, Swift S, Lleres D, Dinkova-Kostova AT. Monitoring Keap1-Nrf2 interactions in single live cells. Biotechnol Adv. 2014;32(6):1133-44.
11. Dikovskaya D, Appleton PL, Bento-Pereira C, Dinkova-Kostova AT. Measuring the Interaction of Transcription Factor Nrf2 with Its Negative Regulator Keap1 in Single Live Cells by an Improved FRET/FLIM Analysis. Chem Res Toxicol. 2019;32:500-512.
12. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al. 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. 2002;99: 11908-13.

13. Dinkova-Kostova AT, Kostov RV, Canning P. Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. Arch Biochem Biophys. 2017;617:84-93.
14. Dayalan Naidu S, Dinkova-Kostova AT. KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease. Open Biol. 2020;10(6):200105.
15. Horie Y, Suzuki T, Inoue J, Iso T, Wells G, Moore TW, et al. Molecular basis for the disruption of Keap1-Nrf2 interaction via Hinge & Latch mechanism. Commun Biol. 2021;4:576.
16. Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, Rumsey WL, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov. 2019;18:295-317.
17. Georgakopoulos N, Talapatra S, Dikovskaya D, Dayalan Naidu S, Higgins M, Gatliff J, et al. Phenyl Bis-Sulfonamide Keap1-Nrf2 Protein-Protein Interaction Inhibitors with an Alternative Binding Mode. J Med Chem. 2022;65:7380-7398.
18. Gorgulla C, Boeszoermenyi A, Wang ZF, Fischer PD, Coote PW, Padmanabha Das KM, et al. An open-source drug discovery platform enables ultra-large virtual screens. Nature. 2020;580:663-668.
19. Lazzara PR, David BP, Ankireddy A, Richardson BG, Dye K, Ratia KM, et al. Isoquinoline Kelch-like ECH-Associated Protein 1-Nuclear Factor (Erythroid-Derived 2)-like 2 (KEAP1-NRF2) Inhibitors with High Metabolic Stability. J Med Chem. 2020;63:6547-6560.
20. Dayalan Naidu S, Suzuki T, Dikovskaya D, Knatko EV, Higgins M, Sato M, et al. The isoquinoline PRL-295 increases the thermostability of Keap1 and disrupts its interaction with Nrf2. iScience. 2020;25:103703.
21. Bertrand HC, Schaap M, Baird L, Georgakopoulos ND, Fowkes A, Thiollier C, et al. Design, Synthesis, and Evaluation of Triazole Derivatives That Induce Nrf2 Dependent Gene Products and Inhibit the Keap1-Nrf2 Protein-Protein Interaction. J Med Chem. 2015;58:7186-94.
22. Du G, Jiang J, Henning NJ, Safaee N, Koide E, Nowak RP, et al. Exploring the target scope of KEAP1 E3 ligase-based PROTACs. Cell Chem Biol. 2022;29:1470-1481.e31.
23. Chen H, Nguyen NH, Magtoto CM, Cobbold SA, Bidgood GM, Meza Guzman LG, et al. Design and characterization of a heterobifunctional degrader of KEAP1. Redox Biol. 2022;59:102552.
24. Davies TG, Wixted WE, Coyle JE, Griffiths-Jones C, Hearn K, McMenamin R, et al. Monoacidic Inhibitors of the Kelch-like ECH-Associated Protein 1: Nuclear Factor Erythroid 2-Related Factor 2 (KEAP1:NRF2) Protein-Protein Interaction with High Cell Potency Identified by Fragment-Based Discovery. J Med Chem. 2016;59:3991-4006.
25. Shin JH, Lee KM, Shin J, Kang KD, Nho CW, Cho YS. Genetic risk score combining six genetic variants associated with the cellular NRF2 expression levels correlates with Type 2 diabetes in the human population. Genes Genomics. 2019;41:537-545.
26. Jia C, Wang R, Long T, Xu Y, Zhang Y, Peng R, et al. NRF2 Genetic Polymorphism Modifies the Association of Plasma Selenium Levels With Incident Coronary Heart Disease Among Individuals With Type 2 Diabetes. Diabetes. 2022;71:2009-2019.
27. Sarutipaiboon I, Settasatian N, Komanasin N, Kukongwiriyapan U, Sawanyawisuth K, Intharaphet P, et al. Association of Genetic Variations in NRF2, NQO1, HMOX1, and MT with Severity of Coronary Artery Disease and Related Risk Factors. Cardiovasc Toxicol. 2020;20:176-189.
28. Shimizu S, Mimura J, Hasegawa T, Shimizu E, Imoto S, Tsushima M, et al. Association of single nucleotide polymorphisms in the NRF2 promoter with vascular stiffness with aging. PLoS One. 2020;15:e0236834.

29. Hua CC, Chang LC, Tseng JC, Chu CM, Liu YC, Shieh WB. Functional haplotypes in the promoter region of transcription factor Nrf2 in chronic obstructive pulmonary disease. Dis Markers. 2010;28:185-93.
30. Sugitani A, Asai K, Watanabe T, Suzumura T, Kojima K, Kubo H, et al. A Polymorphism rs6726395 in Nrf2 Contributes to the Development of Emphysema-Associated Age in Smokers Without COPD. Lung. 2019;197:559-564.
31. Arisawa T, Tahara T, Nakamura M. [A genetic background of ulcer diseases induced by NSAID/aspirin]. Nihon Rinsho. 2010;68:2113-8. (in Japanese).
32. Ji G, Zhang M, Liu Q, Wu S, Wang Y, Chen G, et al. Functional Polymorphism in the NFE2L2 Gene Associated With Tuberculosis Susceptibility. Front Immunol. 2021;12:660384.
33. von Otter M, Landgren S, Nilsson S, Celojevic D, Bergstrom P, Hakansson A, et al. Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease. BMC Med Genet. 2010;11:36.
34. von Otter M, Bergstrom P, Quattrone A, De Marco EV, Annesi G, Soderkvist P, et al. Genetic associations of Nrf2-encoding NFE2L2 variants with Parkinson's disease - a multicenter study. BMC Med Genet. 2014;15:131.
35. Chen YC, Wu YR, Wu YC, Lee-Chen GJ, Chen CM. Genetic analysis of NFE2L2 promoter variation in Taiwanese Parkinson's disease. Parkinsonism Relat Disord. 2013;19:247-50.
36. Gui Y, Zhang L, Lv W, Zhang W, Zhao J, Hu X. NFE2L2 variations reduce antioxidant response in patients with Parkinson disease. Oncotarget. 2016;7:10756-64.
37. Paupe V, Dassa EP, Goncalves S, Auchere F, Lonn M, Holmgren A, Rustin P. Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia. PLoS One. 2009;4:e4253.
38. Quinti L, Dayalan Naidu S, Trager U, Chen X, Kegel-Gleason K, Lleres D, et al. KEAP1-modifying small molecule reveals muted NRF2 signaling responses in neural stem cells from Huntington's disease patients. Proc Natl Acad Sci U S A. 2017;114:E4676-E4685.
39. Nadeem A, Ahmad SF, Al-Harbi NO, Al-Ayadhi LY, Alanazi MM, Alfardan AS, et al. Dysregulated Nrf2 signaling in response to di(2-ethylhexyl) phthalate in neutrophils of children with autism. Int Immunopharmacol. 2022;106:108619.
40. Dinkova-Kostova AT, Kostov RV, Kazantsev AG. The role of Nrf2 signaling in counteracting neurodegenerative diseases. Febs J. 2018;285:3576-3590.
41. Bell KF, Al-Mubarak B, Martel MA, McKay S, Wheelan N, Hasel P, et al. Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. Nat Commun. 2015;6:7066.
42. Cuadrado A, Pajares M, Benito C, Jimenez-Villegas J, Escoll M, Fernandez-Gines R, et al. Can Activation of NRF2 Be a Strategy COVID-19? Trends Pharmacol Sci. 2020;41:598-610.
43. Olagnier D, Farahani E, Thyrsted J, Blay-Cadanet J, Herengt A, Idorn M, et al. SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nat Commun. 2020;11: 4938.
44. van der Horst D, Carter-Timofte ME, van Grevenynghe J, Laguette N, Dinkova-Kostova AT, Olagnier D. Regulation of innate immunity by Nrf2. Curr Opin Immunol. 2022;78:102247.
45. Ordonez AA, Bullen CK, Villabona-Rueda AF, Thompson EA, Turner ML, Merino VF, et al. Sulforaphane exhibits antiviral activity against pandemic SARS-CoV-2 and seasonal HCoV-OC43 coronaviruses in vitro and in mice. Commun Biol. 2022;5:242.

46. Sun Q, Ye F, Liang H, Liu H, Li C, Lu R, et al. Bardoxolone and bardoxolone methyl, two Nrf2 activators in clinical trials, inhibit SARS-CoV-2 replication and its 3C-like protease. Signal Transduct Target Ther. 2021;6:212.
47. Liby KT, Sporn MB. Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. Pharmacol Rev. 2012;64:972-1003.
48. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, et al. Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. Proc Natl Acad Sci U S A. 2005;102:4584-9.
49. Dayalan Naidu S, Muramatsu A, Saito R, Asami S, Honda T, Hosoya T, et al. C151 in KEAP1 is the main cysteine sensor for the cyanoenone class of NRF2 activators, irrespective of molecular size or shape. Scientific Rep. 2018;8:8037.
50. Shekh-Ahmad T, Eckel R, Dayalan Naidu S, Higgins M, Yamamoto M, Dinkova-Kostova AT, et al. KEAP1 inhibition is neuroprotective and suppresses the development of epilepsy. Brain. 2018;141:1390-1403.
51. Liby K, Yore MM, Roebuck BD, Baumgartner KJ, Honda T, Sundararajan C, et al. A novel acetylenic tricyclic bis-(cyano enone) potently induces phase 2 cytoprotective pathways and blocks liver carcinogenesis induced by aflatoxin. Cancer Res. 2008;68:6727-33.
52. Dinkova-Kostova AT, Talalay P, Sharkey J, Zhang Y, Holtzclaw WD, Wang XJ, et al. An exceptionally potent inducer of cytoprotective enzymes: elucidation of the structural features that determine inducer potency and reactivity with Keap1. J Biol Chem. 2010;285: 33747-55.
53. Kostov RV, Knatko EV, McLaughlin LA, Henderson CJ, Zheng S, Huang JT, et al. Pharmacokinetics and pharmacodynamics of orally administered acetylenic tricyclic bis(cyanoenone), a highly potent Nrf2 activator with a reversible covalent mode of action. Biochem Biophys Res Commun. 2015;465:402-7.
54. Knatko EV, Ibbotson SH, Zhang Y, Higgins M, Fahey JW, Talalay P, et al. Nrf2 Activation Protects against Solar-Simulated Ultraviolet Radiation in Mice and Humans. Cancer Prev Res (Phila). 2015;8:475-86.
55. Spencer SR, Wilczak CA, Talalay P. Induction of glutathione transferases and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues. Cancer Res. 1990;50:7871-5.
56. Gopal S, Mikulskis A, Gold R, Fox RJ, Dawson KT, Amaravadi L. Evidence of activation of the Nrf2 pathway in multiple sclerosis patients treated with delayed-release dimethyl fumarate in the Phase 3 DEFINE and CONFIRM studies. Mult Scler. 2017;23:1875-1883.
57. Saito R, Suzuki T, Hiramoto K, Asami S, Naganuma E, Suda H, et al. Characterizations of Three Major Cysteine Sensors of Keap1 in Stress Response. Mol Cell Biol. 2015;36:271-84.
58. Qu L, Guo M, Zhang H, Chen X, Wei H, Jiang L, et al. Characterization of the modification of Kelch-like ECH-associated protein 1 by different fumarates. Biochem Biophys Res Commun. 2022;605:9-15.
59. Sauerland MB, Helm C, Lorentzen LG, Manandhar A, Ulven T, Gamon LF, Davies MJ. Identification of galectin-1 and other cellular targets of alpha,beta-unsaturated carbonyl compounds, including dimethylfumarate, by use of click-chemistry probes. Redox Biol. 2023;59:102560.
60. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, et al. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature. 2018;556:113-117.

61. Zhang Y, Talalay P, Cho CG, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci U S A. 1992;89:2399-403.
62. Traka MH, Saha S, Huseby S, Kopriva S, Walley PG, Barker GC, et al. Genetic regulation of glucoraphanin accumulation in Beneforte broccoli. New Phytol. 2013;198:1085-1095.
63. Armah CN, Traka MH, Dainty JR, Defernez M, Janssens A, Leung W, et al. A diet rich in high-glucoraphanin broccoli interacts with genotype to reduce discordance in plasma metabolite profiles by modulating mitochondrial function. Am J Clin Nutr. 2013;98:712-22.
64. Yagishita Y, Fahey JW, Dinkova-Kostova AT, Kensler TW. Broccoli or Sulforaphane: Is It the Source or Dose That Matters? Molecules. 2019;24(19):3593.
65. Liu H, Zimmerman AW, Singh K, Connors SL, Diggins E, Stephenson KK, et al. Biomarker Exploration in Human Peripheral Blood Mononuclear Cells for Monitoring Sulforaphane Treatment Responses in Autism Spectrum Disorder. Sci Rep. 2020;10:5822.
66. Dinkova-Kostova AT, Fahey JW, Wade KL, Jenkins SN, Shapiro TA, Fuchs EJ, et al. Induction of the phase 2 response in mouse and human skin by sulforaphane-containing broccoli sprout extracts. Cancer Epidemiol Biomarkers Prev. 2007;16:847-51.
67. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol. 2003;23:8137-51.
68. McMahon M, Lamont DJ, Beattie KA, Hayes JD. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. Proc Natl Acad Sci U S A. 2010;107:18838-43.
69. Wang JS, Shen X, He X, Zhu YR, Zhang BC, Wang JB, et al. Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. J Natl Cancer Inst. 1999;91:347-54.
70. de Zeeuw D, Akizawa T, Audhya P, Bakris GL, Chin M, Christ-Schmidt H, et al. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. N Engl J Med. 2013;369:2492-503.
71. Chin MP, Wrolstad D, Bakris GL, Chertow GM, de Zeeuw D, Goldsberry A, et al. Risk factors for heart failure in patients with type 2 diabetes mellitus and stage 4 chronic kidney disease treated with bardoxolone methyl. J Card Fail. 2014;20:953-8.
72. Chertow GM, Appel GB, Andreoli S, Bangalore S, Block GA, Chapman AB, et al. Study Design and Baseline Characteristics of the CARDINAL Trial: A Phase 3 Study of Bardoxolone Methyl in Patients with Alport Syndrome. Am J Nephrol. 2021;52:180-189.
73. Linker RA, Haghikia A. Dimethyl fumarate in multiple sclerosis: latest developments, evidence and place in therapy. Ther Adv Chronic Dis. 2016;7:198-207.
74. Pellacani G, Bigi L, Parodi A, Burlando M, Lanna C, Campione E, et al. Efficacy and Safety of Dimethyl Fumarate in Patients with Moderate-to-Severe Plaque Psoriasis: DIMESKIN-2, a Multicentre Single-Arm Phase IIIb Study. J Clin Med. 2022;11(16):4778.
75. Lynch DR, Farmer J, Hauser L, Blair IA, Wang QQ, Mesaros C, et al. Safety, pharmacodynamics, and potential benefit of omaveloxolone in Friedreich ataxia. Ann Clin Transl Neurol. 2019;6:15-26.
76. Lynch DR, Chin MP, Delatycki MB, Subramony SH, Corti M, Hoyle JC, et al. Safety and Efficacy of Omaveloxolone in Friedreich Ataxia (MOXIe Study). Ann Neurol. 2021;89:212-225.

77. Lynch DR, Chin MP, Boesch S, Delatycki MB, Giunti P, Goldsberry A, et al. Efficacy of Omaveloxolone in Friedreich's Ataxia: Delayed-Start Analysis of the MOXIe Extension. Mov Disord. 2023;38:313-320.
78. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the Hallmarks of Cancer. Cancer Cell. 2018;34(1):21-43.
79. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative Stress in Cancer. Cancer Cell. 2020;38:167-197.
80. DeBlasi JM, DeNicola GM. Dissecting the Crosstalk between NRF2 Signaling and Metabolic Processes in Cancer. Cancers (Basel). 2020;12(10):3023.
81. Torrente L, DeNicola GM. Targeting NRF2 and Its Downstream Processes: Opportunities and Challenges. Annu Rev Pharmacol Toxicol. 2022;62:279-300.
82. Okazaki K, Anzawa H, Liu Z, Ota N, Kitamura H, Onodera Y, et al. Enhancer remodeling promotes tumor-initiating activity in NRF2-activated non-small cell lung cancers. Nature Commun. 2020;11:5911.
83. Sayin VI, LeBoeuf SE, Singh SX, Davidson SM, Biancur D, Guzelhan BS, et al. Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. Elife. 2017;6:e28083.
84. Ghergurovich JM, Garcia-Canaveras JC, Wang J, Schmidt E, Zhang Z, TeSlaa T, et al. A small molecule G6PD inhibitor reveals immune dependence on pentose phosphate pathway. Nat Chem Biol. 2020;16:731-739.
85. Riess JW, Frankel P, Shackelford D, Dunphy M, Badawi RD, Nardo L, et al. Phase 1 Trial of MLN0128 (Sapanisertib) and CB-839 HCl (Telaglenastat) in Patients With Advanced NSCLC (NCI 10327): Rationale and Study Design. Clin Lung Cancer. 2021;22: 67-70.
86. Srivastava R, Fernandez-Gines R, Encinar JA, Cuadrado A, Wells G. The current status and future prospects for therapeutic targeting of KEAP1-NRF2 and beta-TrCP-NRF2 interactions in cancer chemoresistance. Free Radic Biol Med. 2022;192:246-260.
87. Ji J, Ma S, Zhu Y, Zhao J, Tong Y, You Q, Jiang Z. ARE-PROTACs Enable Co-degradation of an Nrf2-MafG Heterodimer. J Med Chem. 2023. doi: 10.1021/acs.jmedchem.2c01909.
88. Okawa H, Motohashi H, Kobayashi A, Aburatani H, Kensler TW, Yamamoto M. Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochem Biophys Res Commun. 2006;339:79-88.
89. Palliyaguru DL, Chartoumpekis DV, Wakabayashi N, Skoko JJ, Yagishita Y, Singh SV, Kensler TW. Withaferin A induces Nrf2-dependent protection against liver injury: Role of Keap1-independent mechanisms. Free Radic Biol Med. 2016;101:116-128.
90. Noh JR, Kim YH, Hwang JH, Choi DH, Kim KS, Oh WK, Lee CH. Sulforaphane protects against acetaminophen-induced hepatotoxicity. Food Chem Toxicol. 2015;80:193-200.
91. Abdelrahman RS, Abdel-Rahman N. Dimethyl fumarate ameliorates acetaminophen-induced hepatic injury in mice dependent of Nrf-2/HO-1 pathway. Life Sci. 2019;217:251-260.
92. Reisman SA, Buckley DB, Tanaka Y, Klaassen CD. CDDO-Im protects from acetaminophen hepatotoxicity through induction of Nrf2-dependent genes. Toxicol Appl Pharmacol. 2009;236:109-14.

93. Kalra S, Zhang Y, Knatko EV, Finlayson S, Yamamoto M, Dinkova-Kostova AT. Oral Azathioprine Leads to Higher Incorporation of 6-Thioguanine in DNA of Skin than Liver: The Protective Role of the Keap1/Nrf2/ARE Pathway. Cancer Prev Res (Phila). 2011;4(10):1665-74.
94. Winski SL, Hargreaves RH, Butler J, Ross D. A new screening system for NAD(P)H:quinone oxidoreductase (NQO1)-directed antitumor quinones: identification of a new aziridinylbenzoquinone, RH1, as a NQO1-directed antitumor agent. Clin Cancer Res. 1998;4:3083-8.
95. Baird L, Suzuki T, Takahashi Y, Hishinuma E, Saigusa D, Yamamoto M. Geldanamycin-Derived HSP90 Inhibitors Are Synthetic Lethal with NRF2. Mol Cell Biol. 2020;40(22):e00377-20.
96. Torrente L, Prieto-Farigua N, Falzone A, Elkins CM, Boothman DA, Haura EB, DeNicola GM. Inhibition of TXNRD or SOD1 overcomes NRF2-mediated resistance to beta-lapachone. Redox Biol. 2020;30:101440.
97. Mansouri A, Reiner Z, Ruscica M, Tedeschi-Reiner E, Radbakhsh S, Bagheri Ekta M, Sahebkar A. Antioxidant Effects of Statins by Modulating Nrf2 and Nrf2/HO-1 Signaling in Different Diseases. J Clin Med. 2022;11(5):1313.
98. Chartoumpekis D, Ziros PG, Psyrogiannis A, Kyriazopoulou V, Papavassiliou AG, Habeos IG. Simvastatin lowers reactive oxygen species level by Nrf2 activation via PI3K/Akt pathway. Biochem Biophys Res Commun. 2010;396:463-6.
99. Liu H, Talalay P. Relevance of anti-inflammatory and antioxidant activities of exemestane and synergism with sulforaphane for disease prevention. Proc Natl Acad Sci U S A. 2013;110:19065-70.
100. Eisenstein A, Hilliard BK, Pope SD, Zhang C, Taskar P, Waizman DA, et al. Activation of the transcription factor NRF2 mediates the anti-inflammatory properties of a subset of over-the-counter and prescription NSAIDs. Immunity. 2022;55:1082-1095.e5.
101. Dinkova-Kostova AT, Copple IM. Advances and challenges in therapeutic targeting of NRF2. Trends Pharmacol Sci. 2023;44:137-149.

KEAP1/NRF2 kao potencijalno ciljno mesto dejstva lekova

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Kratak sadržaj

Nuklearni faktor 2 povezan sa nuklearnim faktorom eritroidom 2 (NRF2, koga kodira *NFE2L2*) je inducibilni transkripcioni faktor koji reguliše ekspresiju široke mreže gena koji kodiraju proteine sa citoprotektivnim funkcijama. NRF2 takođe ima i ulogu u održavanju mitohondrijske i proteinske homeostaze, a njegova aktivacija omogućava adaptaciju na različite vrste ćelijskog stresa. NRF2 je regulisan pre svega na nivou stabilnosti proteina pomoću tri glavna sistema ubikvitin ligaze, pri čemu je najbolje proučena regulacija putem proteina KEAP1, supstratnog adaptera za Cul3/Rbx1-zavisnu ubikvitin ligazu. KEAP1 je multifunkcionalni protein koji, pored toga što je supstratni adapter, predstavlja i senzor za elektrofile i oksidante. Farmakološka inaktivacija proteina KEAP1 ima zaštitni efekat kod životinjskih modela korišćenih za ispitivanje oboljenja koja pogađaju ljude, te je stoga danas prepoznat kao ciljno mesto delovanja lekova, posebno kada je reč o hroničnim oboljenjima kod kojih su oksidativni stres i inflamacija u osnovi patogeneze. Razvijena su brojna jedinjenja usmerena na KEAP1, uključujući elektrofile koji se kovalentno vezuju za cisteinske senzore u KEAP1, neelektrofilne inhibitore protein-protein interakcija koji se vezuju za Kelch domen KEAP1, prekidajući njegovu interakciju sa NRF2, a nedavno i heterobifunkcionalne himere usmerene na proteolizu (PROTACs) koje podstiču proteazomsku razgradnju KEAP1. Razvoj lekova koji sadrže jedinjenja usmerena na KEAP1 doveo je do uvođenja dva jedinjenja, dimetil fumarata (BG-12, Tecfidera®) i RTA-408 (omaveloksolon, SKYCLARYS®), u kliničku praksu. Dimetil fumarat je 2013. odobren kao prvi oralni agens u prvoj liniji lečenja relapsno-remitentne multiple skleroze, a koristi se i u terapiji umerene do jake plak psorijaze. U februaru 2023. godine, američka Uprava za hranu i lekove (FDA) odobrila je omaveloksolon kao prvi i jedini lek namenjen pacijentima obolelim od Fridrajhove ataksije.

Ključne reči: Elektrofil, KEAP1, NQO1, NRF2, omaveloksolon, sulforafan