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Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases

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Abstract

Transcription factor NF-E2 p45-related factor 2 (NRF2, gene name *NFE2L2*) and its principal negative regulator, the E3 ligase adapter Kelch-like ECH-associated protein 1 (KEAP1), play a critical role in the development and progression of chronic diseases of the lung and liver, autoimmune, neurodegenerative and metabolic disorders, and also cancer. NRF2 activation provides cytoprotection against numerous pathologies characterized by chronic inflammation, metabolic alterations and redox disturbances. One NRF2 activator has received clinical approval and several electrophilic modifiers of the cysteine-based sensor KEAP1 and inhibitors of its interaction with NRF2 are now in clinical development. However, challenges regarding target specificity, pharmacodynamic properties, efficacy, and safety remain.

Introduction

Transcription factor NRF2 was discovered in 1994 as a member of the human cap'n'collar (CNC) basic-region leucine zipper transcription factor family¹. Subsequent work, including the generation of NRF2-knockout mice^{2,3}, established that NRF2 regulates the expression of about 250 genes that contain an enhancer sequence in their promoter regulatory regions that is termed the Antioxidant Response Element (ARE). These genes encode a network of cooperating enzymes involved in endobiotic and xenobiotic biotransformation reactions, antioxidant metabolism, intermediate metabolism of carbohydrates and lipids, iron catabolism, protein degradation and regulators of inflammation⁴. Through this transcriptional network, NRF2 is able to coordinate a multifaceted response to diverse forms of stress, enabling maintenance of a stable internal environment.

In 1999, Kelch-like ECH-associated protein 1 (KEAP1) was identified as a repressor of NRF2⁵, and a further 5 years elapsed before it was reported to be an E3 ubiquitin ligase substrate adaptor that targets NRF2 for rapid proteasomal degradation⁶⁻⁸. Thus, KEAP1 ensures that under unstressed conditions NRF2 is a protein of low abundance with a limited half-life of only about 15-40 min, depending on the cell type. Importantly however, KEAP1 contains several highly reactive cysteines that upon modification by electrophilic molecules prevent it from targeting NRF2 for proteasomal degradation, thereby resulting in rapid nuclear accumulation of NRF2 protein upon redox stress and, following dimerization with small musculoaponeurotic fibrosarcoma oncogene homolog (sMAF) proteins, induction of ARE-containing genes by NRF2-sMAF^{9,10}.

It is now widely recognized that because of the versatile and comprehensive cytoprotective roles of the proteins encoded by NRF2 target genes, including anti-oxidant, detoxification and anti-inflammatory proteins, a functional NRF2/KEAP1 axis is essential for protection against a plethora of diseases that have oxidative stress and inflammation as underlying pathological features. These include metabolic and inflammatory/autoimmune disorders, diseases of the lung, liver, kidney, gastrointestinal tract, and cardiovascular system, as well as neurological conditions^{9,11}. The experimental evidence for the protective effects of NRF2 in these non-neoplastic diseases will be discussed later in this review. We will also address the role of NRF2 in cancer, which is a matter of intense research¹².

A three-dimensional reconstruction structure of the KEAP1 dimer obtained by single particle electron microscopy shows that the protein has a forked-stem structure comprising two large spheres that enclose the intervening (IVR), Kelch, and C-terminal-region domains, where dimerization is mediated by the BTB domain¹³ (**Figure 1A**). Bearing in mind that KEAP1 contains a number of distinct and autonomously functioning

cysteine sensors (C151, C226, C273, C288 and C613)¹⁴⁻¹⁶, the existing evidence suggests that NRF2 and KEAP1 integrate nitric oxide, Zn^{2+} and alkenal signaling with redox signaling, and that together they also provide a mechanism that probably evolved, at least in part, to allow rapid adaptation to exposure to phytochemicals encountered in the diet. Many plant-derived electrophilic xenobiotics activate the NRF2/KEAP1 axis to elicit cytoprotective responses, with the highly reactive cysteines in KEAP1 acting as the immediate sensors¹⁷⁻¹⁹. The high reactivity of these sensors with electrophiles allows for therapeutic KEAP1 targeting in the absence of unspecific thiol modifications (**Figure 1B**). Therefore a wealth of naturally-occurring inducers of NRF2-target genes has been described²⁰, and new inducing agents continue to be discovered.

Whilst a substantial number of academic laboratories have been involved in identification of NRF2 activators, historically the engagement of pharmaceutical companies in this exciting and promising field has been relatively low key. This possible reticence of industry to consider the NRF2/KEAP1 axis as a valuable drug target may be due in part to its wide spectrum of biochemical activities, beyond merely controlling antioxidant systems, which are regulated either directly or indirectly by NRF2. The diverse direct and indirect effects of NRF2 activators are sometimes interpreted as evidence for non-specific effects. However, the evolution of several distinct thiol-based redox switches within KEAP1 that allow mammalian species to adapt to a wide spectrum of phytochemicals provides a rationale for changing the perception that activation of NRF2 elicits certain “off-target” effects, and to adopt a broader view that activation of NRF2 elicits beneficial “multi-target” cytoprotective effects¹¹. In particular, the “multi-target” benefits of NRF2 activation include maintenance of redox signaling, enhanced xenobiotic biotransformation, control and resolution of inflammation, suppression of gluconeogenesis and hepatic lipogenesis,

support of proteostasis, and suppression of fibrosis⁹. In this context, it is noteworthy that most chronic diseases, particularly those confined to the aged population, do not have a unique etiology, nor do they exhibit a single pathophenotype, and as such they may be most effectively treated with drugs that activate NRF2. Indeed, stimulation of broad cytoprotective mechanisms upon NRF2 activation is less likely to be effectively overwhelmed or circumvented by pathogenic processes than are more specifically-targeted therapeutic interventions.

This expanded recognition of NRF2 as a potential drug target has sparked the interest of the pharmaceutical industry and led to a substantial investment in the clinical development of NRF2 modulators²¹. The NRF2 activator dimethyl fumarate (DMF, trade names, Fumaderm or Tecfidera) is now clinically used to treat psoriasis and relapsing multiple sclerosis (**Table 1**), and its current distribution remains a hallmark that other pharmaceutical companies hope to achieve. Currently, a number of NRF2 activators of several distinct chemical classes are at various stages of clinical development, such as the KEAP1 cysteine-targeting fumaric acid derivatives, the isothiocyanate sulforaphane, cyanoenone triterpenoids, nitro fatty acids, and hydroxylamine. Additional drug candidates include non-electrophilic compounds that disrupt the NRF2/KEAP1 protein-protein interactions, as well as molecules with KEAP1-independent modes of action. Inhibitors of NRF2 are also being pursued, but are not yet in clinical trials. This review provides an overview of these drug candidates and highlights the unusual nature and the critical importance of context for targeting the NRF2/KEAP1 axis. The physiological roles of NRF2 in cellular redox, metabolic and protein homeostasis, and in resolution of inflammation are described, and experimental evidence for the protective role of NRF2 in non-neoplastic disease is presented. The advantages and limitations of the various small

molecule pharmacological modulators of NRF2 activity that are in clinical development are assessed. Finally, ongoing challenges associated with the development of NRF2 modulators, including target specificity, pharmacodynamics assessment, bioavailability, efficacy, and safety are highlighted.

Physiological roles of the NRF2/KEAP1 axis

Redox, metabolic and protein homeostasis

Since its initial discovery in the participation of biotransformation reactions, in which it regulates the expression of certain cytochrome P450 oxidoreductases, conjugating enzymes and ABC transporters (**Table 2**), it has become increasingly apparent that NRF2 is involved in control of the redox environment, culminating in recognition of the NRF2/KEAP1 axis as a ‘thiol-driven master switch’ that is used for ‘system-wide oxidative stress responses’²². NRF2 regulates the expression of the four genes (glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme 1, and isocitrate dehydrogenase 1) involved in the generation of NADPH, the critical cofactor that fuels antioxidant reactions²³⁻²⁶. The supply of this reducing equivalent is further used by a plethora of redox reactions, many of which are also regulated by NRF2. Thus, NRF2 regulates the expression of critical enzymes involved in the synthesis and use of the redox buffer reduced glutathione (GSH, the oxidized form of which is GSSG), such as the catalytic and modulator subunits of glutamate-cysteine ligase, glutathione reductase, glutathione peroxidase and several glutathione *S*-transferases⁴. Moreover, many ancillary proteins within the redoxin family such as thioredoxin, thioredoxin reductase, peroxiredoxin and sulfiredoxin, are all regulated by NRF2, and provide compartmentalized sensing and signal transduction of regional production of reactive oxygen species (ROS, i.e., hydrogen

peroxide, the hydroxyl radical and the superoxide radical)²⁷⁻²⁹. Besides these direct effects on redox status, NRF2 regulates genes encoding enzymes that prevent quinones from participating in redox cycling reactions and glutathione depletion (by NAD(P)H:quinone oxidoreductase-1 (NQO1)) or are involved in the indirect production of bilirubin (heme oxygenase 1, HMOX1; biliverdin reductase, BVR), which is the most potent non-polar physiological antioxidant³⁰. Finally, through crosstalk with the pentose phosphate pathway and glycolysis, NRF2 affects intermediary metabolism and increases the availability of substrates and reducing equivalents for the mitochondrial respiratory chain³¹, as well as for maintaining integrity of mitochondrial DNA (mtDNA)³².

NRF2 participates in the clearance of oxidized, or otherwise damaged, proteins and organelles during redox alterations or nutritional starvation. Thus, the autophagy transporter sequestosome 1 (SQSTM1/p62), which interacts with ubiquitinated cargo via its ubiquitin association (UBA) domain and recruits them into the autophagosome via its LC3-interacting motif, is an NRF2 transcriptional target^{33,34}. In addition, KEAP1 binds to p62^{33,35}, and a recent study has found that KEAP1/Cul3 ubiquitinates p62 at lysine-420 within its UBA domain, thereby facilitating the sequestration activity of p62³⁶. Within its primary structure, p62 contains an ETGE-like motif, STGE, which upon phosphorylation by mTORC1 generates the recognition site for docking to KEAP1³⁷. Then, KEAP1 is transported to the autophagosome for degradation, thus allowing accumulation of NRF2³⁸. Moreover, several autophagy genes appear to contain ARE sequences in their promoter regions, and it has been reported that NRF2 activates both chaperone-mediated autophagy³⁹ and macroautophagy^{40,41}. Other roles of NRF2 in proteostasis under oxidative conditions are demonstrated by its activation by the unfolded protein response^{42,43} and its ability to regulate proteasome subunit expression^{44,45}.

At least in brain and blood, the abundance of mRNA encoding NRF2 is higher than that for KEAP1 (**Figure 2**) and by contrast the half-life of the KEAP1 protein is longer than that of NRF2 (**Table 2**). These observations highlight the different turnover rates of both proteins: slow for KEAP1; fast for NRF2. Moreover, although NRF2 and KEAP1 are ubiquitously expressed, specific cell types are predominantly in charge of homeostatic adaptations and present different levels of expression (**Figure 2**). Thus, monocytes and neutrophils exhibit the highest levels of NRF2 among blood cells, suggesting an immunomodulatory effect of the innate immune system (see below). In the brain, crosstalk between astrocytes and neurons couples intermediate metabolism with redox homeostasis, at least in part through NRF2⁴⁶. Moreover, glutamatergic neurotransmission in neurons leads to production of ROS that are controlled by the neuronal GSH pool. When this pool is low, neighboring astrocytes contribute to its restoration by providing neurons with the GSH precursors glycine, glutamate/glutamine and cysteine⁴⁷. Not surprisingly, NRF2 levels are high in astrocytes (**Figure 2**). Moreover, microglia as part of the monocyte lineage expresses high levels of NRF2 compared to neurons.

Resolution of inflammation

NRF2 is abundant in monocytes and granulocytes (Figure 2), suggesting its involvement in immune responses driven by these cell types. NRF2-knockout mice are hypersensitive to septic shock⁴⁸, and display persistent inflammation during wound healing^{49,50}, whereas genetic or pharmacological activation of NRF2 suppresses production of pro-inflammatory cytokines⁵¹⁻⁵⁶. In human cells, disruption of KEAP1, protected against sepsis by attenuating the inflammatory response of myeloid leukocytes⁵⁷ and macrophages⁵⁸. Of particular interest, a polymorphism in *NFE2L2* that is associated with reduced transcriptional activity

correlated with increased risk of inflammatory bowel disease⁵⁹ and chronic gastritis⁶⁰. As indicated below, the robust anti-inflammatory activity of NRF2 has been attributed to at least three independent mechanisms: 1, modulation of redox metabolism; 2, crosstalk with nuclear factor-kappaB (NF- κ B); 3, direct regulation of pro-inflammatory genes.

Inflammation is associated with increased local and systemic accumulation of pathological levels of ROS that may impair redox signaling⁶¹. Mitochondrial dysfunction and uncontrolled activation of NADPH oxidase represent the main contributors to heightened ROS production in inflammatory cells, and mitochondrial ROS cause damage and release of mtDNA, thus creating a vicious cycle of events leading to further ROS production and activation of the inflammasome, ultimately resulting in organ debilitation⁶². The redox-regulating activity of NRF2 represents an important break on the vicious cycle that prevents exacerbated inflammation and subsequent tissue damage⁶³.

NF- κ B and NRF2 engage in crosstalk. Several functional NF- κ B binding sites have been identified in the *NFE2L2* gene promoter⁶⁴. On the other hand, NRF2 inhibits NF- κ B transcriptional activity. Treatment of NRF2-deficient mice with lipopolysaccharide (LPS) or TNF- α increased the expression of the NF- κ B transcriptional signature, suggesting an inhibitory role of NRF2 on NF- κ B⁴⁸. A possible interpretation of this finding is that the inhibitor of NF- κ B (I κ B) is highly phosphorylated in NRF2-deficient cells and subject to rapid proteasomal degradation, thereby diminishing inhibition of NF- κ B⁴⁸. Additionally, LPS was shown to activate simultaneously both a fast NF- κ B-mediated response and a slow NRF2-mediated response, with the initial pro-inflammatory response being driven by NF- κ B which was then subsequently inhibited when NRF2 activity was maximal⁶⁵. Other mechanisms by which the NRF2/KEAP1 axis suppresses NF- κ B signaling include

competition between NRF2 and p65 for binding to p300⁶⁶ and degradation of IκB kinase (IKK)β by KEAP1^{67,68}.

Several macrophage-specific genes contain ARE sequences and are therefore regulated by NRF2. These include MARCO, a receptor required for bacterial phagocytosis, and CD36, a scavenger receptor for oxidized low-density lipoproteins^{69,70}. On the other hand, NRF2 binds to regulatory regions of pro-inflammatory genes encoding IL-6 and IL-1β in an ARE-independent manner and prevents the recruitment of RNA Pol II to start transcription⁵².

Recent studies have provided a connection between macrophage metabolism and anti-inflammatory responses elicited by NRF2. The mitochondrial metabolite itaconate is produced from the TCA cycle in response to pro-inflammatory stimuli and increases profoundly during macrophage activation^{71,72}. In turn, itaconate alkylates several KEAP1 cysteine sensors, including the critical C151, C273 and C288 residues, thereby activating NRF2 and suppressing transcription of pro-inflammatory cytokines, such as IL-6 and IL-1β⁷¹. Therefore, itaconate is an endogenous regulator of KEAP1 that acts as an ‘off-switch’ during the resolution of inflammation. In addition, activation of NRF2 by the cell-permeable itaconate derivative, 4-octyl-itaconate or sulforaphane represses the expression of the adaptor molecule stimulator of interferon genes (STING) by decreasing its mRNA stability, and consequently suppresses type-I interferon production^{73,74}.

The NRF2/KEAP1 axis in non-neoplastic disease

A wealth of information has been generated in cellular and animal models about the role of NRF2 in providing protection against numerous chronic diseases, with many investigations

employing NRF2-knockout mice⁷⁵. Regarding humans, a recent study provided a basis to analyze the contribution of NRF2 in combatting chronic disease using a Systems Medicine approach and, based on the connectivity of NRF2 with other molecules (NRF2-interactome) along with empirical evidence, established a first map of NRF2-related diseases (NRF2-diseasome)⁹. Compelling evidence for a role of NRF2 in determining the susceptibility of humans to chronic disease is continually being provided by association studies between functional genetic variations of *NFE2L2* and disease risk⁷⁶⁻⁷⁸. Moreover, these genetic studies support the hypothesis that drugs mimicking small changes in NRF2 expression reported for some functional variations of *NFE2L2* may be of therapeutic value. Below we will briefly describe some milestones in understanding the involvement of NRF2 in human chronic disease and highlight the available genetic evidence.

Autoimmune disease

Oxidative tissue damage and apoptosis lead to the formation of haptens or damaged macromolecules that increase the risk of autoimmune reactions. Since NRF2-regulated enzymes play a critical role in detoxification of many chemicals, it provides a protective mechanism against the environmental susceptibility to autoimmune pathogenesis⁷⁹. Moreover, NRF2 suppresses pro-inflammatory Th1 and Th17 responses and activates immunosuppressive Treg and Th2 cells⁸⁰. The relevance of NRF2 in autoimmunity has been extensively studied in experimental autoimmune encephalomyelitis (EAE), which is a mouse model for MS, a chronic inflammatory disease characterized by infiltration of autoreactive immune cells into the central nervous system. In an EAE mouse model, the absence of NRF2 exacerbates disease⁸¹ whereas knockdown of KEAP1⁵² or treatment with a range of NRF2 activators, including cyanoenone triterpenoids⁸² and sulforaphane⁸³,

attenuate its severity. In humans, gene expression profiling in IFN-1 β -treated patients identified NRF2 as a potential mediator of long-term antioxidant response and neuronal preservation⁸⁴. As described below, several biopharmaceutical companies are exploiting the strong evidence that activation of NRF2 is of therapeutic value in MS. Indeed, the only NRF2 activator so far approved by the FDA and the EMA, dimethyl fumarate, is being marketed by Biogen for the treatment of relapsing remitting MS and psoriasis⁸⁵. A protective role for NRF2 has been suggested in other autoimmune diseases such as systemic lupus erythematosus^{86,87}, Sjogren's syndrome⁸⁶, rheumatoid arthritis^{88,89}, vitiligo⁹⁰⁻⁹², and STING-dependent interferonopathies⁷⁴.

Respiratory disease

The lung responds to a variety of environmental toxicants, including smoke, by releasing pro-inflammatory cytokines and chemokines. This primary effect generates a progressive low-grade inflammatory response through the recruitment of circulating monocytes, neutrophils and T cells that release additional inflammatory mediators, ROS and metalloproteases. The end result is a vicious cycle of damage to lung parenchyma that leads to chronic obstructive pulmonary disease (COPD) or emphysema⁹³. NRF2 attenuates the burden to lung parenchyma caused by ROS and inflammation. Cigarette smoke is a major risk factor for the development of COPD and emphysema.

Nrf2^{-/-} mice are much more sensitive to the development of cigarette smoke-induced emphysema than their wild-type counterparts⁹⁴. Furthermore, the development of emphysema following chronic exposure to cigarette smoke was associated with a decline in the expression of cytoprotective genes⁹⁵, whereas the pentacyclic cyanoenone CDDO-imidazolidine protected the lungs of *Nrf2*^{+/+}, but not *Nrf2*^{-/-} mice, against cigarette smoke-

induced oxidative stress, alveolar cell apoptosis, alveolar destruction, and pulmonary hypertension⁹⁶. Genetic upregulation of NRF2 (by *Keap1* deletion in Clara cells, which are abundant in murine upper airways) has a similar protective effect⁹⁷.

In humans, the NRF2 transcriptional signature was decreased in alveolar macrophages from patients with smoking-related lung emphysema as compared with patients without emphysema⁹⁸. The human *NFE2L2* gene promoter contains a haplotype comprising 3 single nucleotide polymorphism (SNP) sequences and 1 triplet repeat polymorphism that result in modulation of NRF2 expression. COPD patients with low to medium promoter activity were more prone to developing respiratory failure⁹⁹. Pharmacological activation of NRF2 by sulforaphane, or a compound that disrupts the interaction of KEAP1 with NRF2, reversed the impaired bacterial phagocytosis by cultured alveolar macrophages and monocyte-derived macrophages isolated from patients with COPD^{70,100}. However, daily oral administration of sulforaphane (extracted from broccoli sprouts) to COPD patients did not result in consistent changes in NRF2-dependent gene expression or markers of inflammation in alveolar macrophages and bronchial epithelial cells at the doses used¹⁰¹, illustrating some of the challenges in translating findings from cell culture studies to humans. Patients with other chronic lung diseases, such as idiopathic pulmonary fibrosis, chronic sarcoidosis and hypersensitivity pneumonitis, were found to exhibit increased NRF2 expression and augmented levels of endogenous antioxidants in bronchoalveolar lavage fluids, suggesting an unsuccessful adaptive response to pathological ROS levels¹⁰².

In agreement with the human data, *Nrf2*^{-/-} mice are much more sensitive to the development of cigarette smoke-induced emphysema than their wild-type counterparts⁹⁴. Furthermore, the development of emphysema following chronic exposure to cigarette

smoke was associated with a decline in the expression of cytoprotective genes⁹⁵, whereas the pentacyclic cyanoenone CDDO-imidazolidine protected the lungs of *Nrf2*^{+/+}, but not *Nrf2*^{-/-} mice, against cigarette smoke-induced oxidative stress, alveolar cell apoptosis, alveolar destruction, and pulmonary hypertension⁹⁶. Genetic upregulation of NRF2 (by *Keap1* deletion in Clara cells, which are abundant in murine upper airways) has a similar protective effect⁹⁷.

Gastro-intestinal disease

The gastro-intestinal (GI) tract is constantly exposed to xenobiotic challenges that can lead to formation of pathological levels of ROS and may provoke inflammatory bowel disease (IBD)¹⁰³. The GI tract is also one of the sites where pharmacological NRF2 activation by dietary agents and their synthetic analogs is highly prominent^{23,104-106}. In mice, dextran sulfate sodium-induced experimental colitis elicits more profound IBD in NRF2-deficient mice than in their wild-type counterparts¹⁰⁷. In humans, the relevance of NRF2 in adaptation of enterocytes to inflammatory stress was demonstrated in a comprehensive transcriptome study on IBD patients that evidenced its role in attenuation of the stress response of macrophages in the gut¹⁰⁸. Importantly, a genetic association has been found in a Japanese cohort of patients between a particular SNP in the *NFE2L2* gene and the risk of developing ulcerative colitis¹⁰⁹. The functional haplotype of the three SNPs in the *NFE2L2* promoter with slightly reduced expression of NRF2 was associated with development of gastric mucosal inflammation and peptic ulcer in association with *H. pylori* infection⁶⁰.

The liver represents a first line of defense against food xenobiotics and orally-active drugs because a major portion of blood draining the GI tract is directed to the liver via the hepatic portal vein. Early work with *Nrf2*^{-/-} mice demonstrated the hepatoprotective effect

of NRF2 against acetaminophen¹¹⁰, as did genetic activation of NRF2¹¹¹. As possibly anticipated, *Nrf2*^{-/-} mice fed alcohol (4-7 days) are markedly more susceptible to fatty liver aggravated hepatic inflammatory responses and liver failure than are similarly fed wild-type mice, and this is in part likely due to activation of the SREBP lipogenic transcription factor and a relative inability to detoxify acetaldehyde¹¹². The observation that disruption of NRF2 in mice impeded liver regeneration following partial hepatectomy led to the discovery that NRF2 regulates the gene expression of Notch1¹¹³, a mechanism that is also involved in the NRF2-mediated improvement of hematopoietic stem progenitor cell function and myelosuppression following exposure to ionizing radiation¹¹⁴. In a mouse model of hereditary hemochromatosis with iron overload (due to mutation of the Homeostatic Iron Regulator gene), knockout of NRF2 increased necro-inflammatory lesions within livers of the knockout mice that led to hepatic fibrosis¹¹⁵. Consistent with these observations, patients with primary biliary cholangitis and cirrhosis exhibit reduced NRF2 expression¹¹⁶.

Metabolic disease

Metabolic disease, also referred to as metabolic syndrome, is typified by abdominal obesity, hypertension, hypertriglyceridemia, low high-density lipoprotein and fasting hyperglycemia. It is closely linked with type-2 diabetes mellitus (T2DM) and associated comorbidities such as vascular dysfunction, cardiovascular disease, glomerulonephritis, NASH (see above) and cognitive impairment. Although T2DM and its comorbidities are characterized by overproduction of superoxide by mitochondria¹¹⁷, NRF2-null mice do not spontaneously develop diabetes. Indeed, the knockout mice exhibit increased insulin sensitivity, which has been attributed to ROS-mediated inhibition of protein tyrosine kinase

phosphatase 1B that antagonizes insulin signaling¹¹⁸. By contrast, genetic activation of NRF2 by knockout/knockdown of KEAP1 in mice on a *db/db* background, or pharmacological activation of NRF2 in *db/db* mice, suppress the onset of T2DM¹¹⁹. It therefore seems likely that when pancreatic beta cells are metabolically challenged to synthesize insulin in response to hyperglycemia, the resulting ROS burden may cause oxidative damage that impairs the response, leading to T2DM^{120,121}. If this conclusion is correct, it is likely that improved pancreatic function contributes significantly to the improved glucose disposal observed following pharmacological activation of NRF2 by TBE-31 or sulforaphane in mice with diet-induced T2DM^{122,123}, and in the reduction of fasting blood glucose and glycated hemoglobin (HbA1c) by sulforaphane-rich broccoli sprout extracts in obese patients with dysregulated T2DM¹²³. Besides imparting increased resilience to oxidative stress on pancreatic beta cells, NRF2 activation may contribute to improved whole-body glucose homeostasis by suppressing glycogen breakdown in skeletal muscle through increasing expression of glycogen branching enzyme and phosphorylase *b* kinase α ¹¹⁹. In mouse models of diabetic nephropathy, pharmacological activation of NRF2 by sulforaphane or cinnamic aldehyde has been shown to suppress oxidative stress, transforming growth factor- β 1 signaling and fibrosis¹²⁴.

It seems likely that NRF2 plays a key role in insulin resistance as suggested by the fact that its expression is low in monocytes of diabetic patients¹²⁵. Compelling evidence comes from genetic association studies indicating that some *NFE2L2* polymorphisms that result in low NRF2 expression lead to pathological ROS levels and increased risk of T2DM¹²⁶⁻¹²⁸. Regarding complications, a failure of antioxidant protection due to

demethylation of the CpG islands in the *KEAP1* promoter has been linked to diabetic cataracts¹²⁹.

NRF2 may also be protective against the development of nonalcoholic steatohepatitis (NASH). In a mouse model of NASH that involves administration of a methionine- and choline-deficient diet, NRF2-null mice succumbed to the disease much more rapidly than did wild type mice^{130,131}. In a more physiological model of NASH, NRF2-deficient mice fed a high-fat diet also developed exacerbated hepatic steatosis and inflammation suggesting the need for NRF2 to suppress the onset of these symptoms^{118,132}. Furthermore, pharmacologic activation of NRF2 by the tricyclic cyanoenone TBE-31 in wild-type, but not NRF2-knockout mice, which had been rendered obese and insulin resistant by chronic consumption of high-fat and high-fructose diet, reversed insulin resistance, suppressed hepatic steatosis, and ameliorated both NASH and liver fibrosis¹²². Pharmacological activation of NRF2 by TBE-31 antagonizes SREBP-1c and ChREBP and expression of their lipid-biosynthetic target genes, which likely involves activation of AMPK^{122,133}. Moreover, in mouse liver, genetic activation of NRF2 antagonizes expression of the gluconeogenic enzymes PEPCK and G6PC, which may also involve AMPK¹³³. In liver biopsies of NASH patients, pathological ROS levels were found together with an increased NRF2-gene signature, suggesting an attempt to reduce the oxidant and inflammatory burden¹³⁴.

Cardiovascular disease

An imbalance between the production and disposal of ROS resulting in an excessive formation of damaging oxidative species has been postulated to play a role in a number of cardiovascular diseases, such as hypertension, atherosclerosis, diabetic vascular disease,

myocardial ischemia-reperfusion injury and heart failure¹³⁵. In addition, low grade, persistent inflammation within the vascular wall is characteristic of the atherogenic process and development of vulnerable atherosclerotic plaques that are prone to rupture, subsequently leading to thrombosis and end-organ ischemia. Given that NRF2 is the key regulator of antioxidant defense and also has direct anti-inflammatory properties, it is logical to assume that NRF2 would protect against ROS-related cardiovascular disease. Indeed, NRF2 activating agents, including fumarate and hydrogen sulfide, have shown protective effects in many animal models of cardiovascular disease, such as cardiac ischemia-reperfusion injury^{136,137} and heart failure^{138,139}. The role of NRF2 in atherosclerosis in mouse models of hypercholesterolemia is less straightforward: the loss of NRF2 in bone marrow derived cells aggravates atherosclerosis^{140,141}, whereas global deficiency of NRF2 alleviates atherosclerosis assessed by the extent of lesion development¹⁴²⁻¹⁴⁵, yet increases plaque inflammation and vulnerability¹⁴⁵. Nevertheless, several NRF2 activating agents have been shown to be atheroprotective in a variety of animal models of atherosclerosis¹⁴⁶⁻¹⁴⁸. Cardiac diseases caused by mutations in contractile, cytoskeletal proteins and molecular chaperones can account for about 30% of inheritable cardiomyopathies¹⁴⁹. In some of these settings, reductive rather than oxidative stress, coupled with protein aggregation, is associated with the cardiomyopathies. Recently, systemic inflammation and pathological ROS formation in hemodialysis patients were associated with down-regulation of NRF2¹⁵⁰ and a polymorphism located in the *NFE2L2* promoter has been associated with increased mortality in these patients¹⁵¹. Disruption of the NRF2 signaling axis prevents reductive stress and delays proteotoxic cardiac disease in mice overexpressing human CRYAB (α -crystalline B chain), a molecular chaperone, in the

heart¹⁵². Thus, as highlighted throughout this review, the context for targeting NRF2 is very important – more may not always be better.

Neurodegenerative disease

In various forms of neurodegenerative disease, the connection between NRF2 and proteostasis is particularly pertinent since these diseases are characterized by abnormal protein aggregation⁴³. Compelling evidence for a protective role of NRF2 in proteinopathy has been provided in Alzheimer's disease (AD) models of amyloidopathy^{153,154}, tauopathy¹⁵⁵ or both^{40,156,157} and in Parkinson's disease (PD) models of alpha-synucleinopathy¹⁵⁸⁻¹⁶⁰. In humans, APP and TAU injured neurons express increased levels of NRF2 and its target p62^{40,161}. These findings are consistent with the recently reported role of NRF2 in upregulating expression of genes involved in macroautophagy⁴⁰ and chaperone mediated autophagy³⁹, two essential mechanisms for clearance of APP, TAU and α -synuclein. Upregulation of NRF2 protected neurons against the toxicity of mutant α -synuclein and leucine-rich repeat kinase 2 (LRRK2)¹⁶², which also leads to neurodegeneration associated with accumulation of misfolded proteins¹⁶³. Curiously, whereas NRF2 activation increased the degradation of α -synuclein, misfolded diffuse LRRK2 was sequestered into inclusion bodies.

The role of NRF2 in astrocytes and microglia is expected to be especially relevant as they show the highest expression levels in the brain (**Figure 2B**). Using transgenic mice with an ARE-driven alkaline phosphatase reporter gene, it was found that astrocytes are highly responsive to NRF2 activation in several models of neurodegeneration¹⁶⁴, pointing to the relevance of NRF2 in the nurturing effect of astrocytes by providing metabolic and GSH precursors to endangered neurons^{46,165}. Microglia, as the immune response cell of the

brain, is also crucial in NRF2 mediated responses^{166,167}. In the murine model of PD based on intoxication with the Parkinsonian toxin methyl-4-phenyl-1,2,3,6-tetrahydropyridine, it was reported that NRF2 modulates microglial dynamics, and thereby reduces production of COX-2, NOS2, IL-6, and TNF- α and increases the levels of several anti-inflammatory markers^{55,168}. The activation of microglia by TAU-injured neurons appears to be related, at least in part, to the crosstalk through the chemokine fractalkine. Both in mice and in humans, TAU-injured neurons release fractalkine, which in neighboring microglia inhibits GSK-3 and leads to increases in NRF2 protein. In turn, NRF2 reduces the release of TNF- α and IL-6 and participates in the reprogramming of microglia towards a wound healing response¹⁶¹.

Several NRF2-target genes including *HMOX1*, *NQO1*, *GCLM* and *p62/SQSTM1* are upregulated in AD and PD brains^{159,169-171}. The relevance of this upregulation is demonstrated by genetic analyses of the association between disease risk and the functional haplotype made of three SNPs in the *NFE2L2* promoter. In ALS, a protective haplotype allele was associated with a 4-year delay in disease onset¹⁷², but another study did not find a clear association¹⁷³. In AD, another haplotype variant was associated with a 2-years earlier onset of disease⁷⁶. More detailed evidence has been reported for PD. Initially, a protective haplotype was associated with delayed onset of disease in a Swedish cohort and reduced risk in a Polish cohort of PD patients⁷⁷. Afterwards, these findings were replicated in four independent European case-control studies¹⁷⁴, but not in a Taiwanese population¹⁷⁵, suggesting differences in ethnicities and environmental factors.

Pharmacological activation of NRF2 also holds promise for the treatment of Huntington's disease (HD) and Friedreich's ataxia (FRDA), where oxidative stress and inflammation are important drivers of pathology. Studies in mice, model organisms and

cultured cells from patients with HD and FRDA have reported impaired NRF2 signaling^{53,176-178}, and the beneficial effects of pharmacological NRF2 activators. Thus, activation of NRF2 by triazole derivatives suppressed the release of pro-inflammatory cytokines in primary mouse HD microglia and astrocytes and in cultured monocytes from HD patients⁵³, and was neuroprotective in *ex vivo* HD rat corticostriatal brain slices and an HD Drosophila model¹⁷⁹. In mouse models of HD, DMF and the triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improved the brain pathology and behavior of the animals^{180,181}. Treatment with sulforaphane and the cyclic cyanoenones TBE-31 or RTA-408 led to improved mitochondrial function and protection against oxidative stress in fibroblasts and cerebellar granule neurons from FRDA mouse models, and in fibroblasts from HD patients^{182,183}. Altogether, the available evidence suggests that a modest activation of NRF2 is neuroprotective in the brain.

The NRF2/KEAP1 axis and cancer

NRF2 has been reported to exert both anti- and pro-tumorigenic actions. Here we provide a brief summary of the current understanding of the role of NRF2 in cancer and refer the reader to a recent comprehensive review discussing NRF2 in the context of the hallmarks of cancer¹².

In non-malignant cells, NRF2 activation affords resistance to oxidant-induced genetic damage and chemical and physical carcinogens due to enhanced defenses that include antioxidant¹⁸⁴ and radioprotective¹¹⁴ activities, as well as accelerated biotransformation and clearance of DNA-damaging agents¹⁸⁵. Moreover, activation of the NRF2 transcriptional response appears to maintain ROS levels below those necessary for signaling to proteins critical for tumorigenesis, such as phosphatidylinositol-4,5-

bisphosphate 3-kinase (PI3K), mitogen activated kinases, hypoxia inducible factor-1 and NF- κ B¹⁸⁶.

On the contrary, in the early stages of tumor development, cancer cells with constitutive activation of NRF2 are selected as a means of enabling adaptation to a hostile microenvironment, chemotherapy, radiotherapy or high endogenous ROS levels. Additionally, in rapidly proliferating cells, NRF2 supports intermediary metabolism by enhancing the biosynthesis of nucleotides²⁶ and amino acids¹⁸⁷, but also generates metabolic imbalance and dependence on glutaminolysis, which could be therapeutically exploited^{188,189}.

Somatic loss-of-function mutations in *KEAP1* or gain-of-function mutations in *NFE2L2* are common in non-small cell lung cancer and some other cancers in which environmental factors are important etiological components¹⁹⁰. A comprehensive catalogue of NRF2 mutations in The Cancer Genome Atlas (TCGA) database identified 226 unique NRF2-mutant tumors within 10,364 cases, with gain-of-function NRF2 mutations occurring in 21 out of 33 tumor types¹⁹¹. The frequent occurrence of such mutations suggests a potential benefit of NRF2 inhibitors in cancer treatment, triggering a search for NRF2 inhibitors (**Box 1**). This is a completely open field for pharmacology and at this time is at the level of proof-of-concept research. Alternative approaches for NRF2 suppression include targeting proteins that confer dependence on NRF2 and/or are selectively expressed in KEAP1-mutant cancer cells. Examples include inhibitors of glutaminase^{188,189} and NR0B1, an atypical orphan nuclear receptor that participates in a multimeric protein complex to regulate transcription in KEAP1-mutant cells¹⁹². It should be noted however that no evidence exists to date that either *KEAP1* or *NFE2L2* mutation alone can instigate malignant transformation, but co-occurrence with oncogenic drivers seems necessary; PI3K

kinase pathway alterations having the strongest association¹⁹³. Most tellingly, the hypomorphic “*Keap1*-knockdown” mouse in which NRF2 is globally upregulated does not spontaneously develop tumours^{194,195}, whereas the simultaneous deletion of *Keap1* and *Pten* in the murine lung promotes adenocarcinoma formation¹⁹⁵. Importantly, the resulting tumors are characterized by an immunosuppressive microenvironment, which can be therapeutically exploited by use of immune checkpoint inhibitors¹⁹⁵.

It is clear that the context within which NRF2 is genetically activated is important in terms of tumorigenesis, as oncogene (K-Ras^{G12D}) activation of NRF2 signaling in rodents can enhance tumor development¹⁹⁶ whereas genetic knockdown of *Keap1* (also leading to activation of NRF2 signaling) strongly blunts Notch1-driven tumorigenesis¹⁹⁷ as well as UV radiation-mediated cutaneous carcinogenesis^{51,198}. Notably, in the latter model, the protective effect of KEAP1 knockdown against initiation of cutaneous carcinogenesis is abrogated by the simultaneous loss of NRF2¹⁹⁹. This dichotomous role of NRF2 is supported by extensive experimental evidence in which its activation enhances antitumor immunity to prevent lung carcinogenesis, but only after tumor initiation accelerates malignant growth²⁰⁰. Also, *Nrf2*-knockout mice submitted to chemically-induced carcinogenesis exhibit an increased number of tumors (enhanced initiation) but of much smaller size (impaired progression) compared to wild-type littermates²⁰¹. Hence, low NRF2 activity facilitates initiation of carcinogenesis, whereas persistent constitutively high NRF2 activity can drive cancer progression and resistance to therapy²⁰².

NRF2 modulators in clinical development

Fumaric acid esters

Fumaric acid esters represent a group of NRF2 activators upon which industry is focusing huge effort. The most clinically successful example is DMF (compound **1**, **Figure 3A**), which was approved in 1994 for the treatment of psoriasis and, based on its efficacy in the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis²⁰³, was repurposed in 2014 by Biogen (Tecfidera) for the treatment of relapsing-remitting multiple sclerosis. Early studies in rodents preceding the discovery of NRF2 had reported that DMF is a robust inducer of the NRF2-transcriptional targets GST and NQO1²⁰⁴, and it was later shown that the DMF metabolite monomethyl fumarate (MMF, compound **2**, **Figure 3A**) reacts with C151 in KEAP1, thereby activating NRF2²⁰³. Safety has been a priority since the discovery of the inducer activity of DMF, and it was noted that the concentrations of DMF required to obtaining significant enzyme inductions were well tolerated by rodents²⁰⁴. DMF exhibited a favorable safety and tolerability profile in two phase 3 trials^{205,206}, and after its commercialization it has become one of the most successful new medicines in recent years. However, a drawback of Tecfidera is the occurrence of mild to moderate abdominal pain, flushing, diarrhea and nausea. While these adverse effects can be managed, a more serious symptom is the occurrence of leukopenia in patients with low leukocyte counts at the beginning of treatment. In fact, animals treated with DMF had much lower levels of granulocytes in their nervous system than those that did not receive the drug²⁰⁷. This effect is not related to NRF2 but is most likely due to activation of the hydroxycarboxylic acid receptor since mice lacking this protein had high levels of granulocytes regardless of whether they were treated with DMF²⁰⁷. These observations highlight the importance of carefully selecting the inclusion criteria for treatment.

As mentioned above, DMF is metabolized *in vivo* to MMF, which inactivates KEAP1 through adduct formation at C151²⁰³. Because of this metabolic conversion, several

biopharmaceutical companies are now developing compounds with slow and sustained release of MMF that would show improved bioavailability and fewer side effects compared to DMF (**Table 1**). Alkermes (acquired by Biogen) has developed diroximel fumarate (ALK8700/BII089, compound **3**, **Figure 3A**), an MMF prodrug with reduced gastrointestinal side effects that is now in a phase 3 clinical trial for multiple sclerosis (NCT03093324). XenoPort (acquired by Arbor Pharmaceuticals) has developed tepilamide fumarate, an MMF prodrug (XP23829, compound **4**, **Figure 3A**). Compared to DMF, XP23829 has higher solubility and permeability, greater absorption following oral administration, improved efficacy and reduced gastrointestinal side effects in preclinical models, and is now in a phase 2 clinical trial for plaque psoriasis (NCT02173301). Catabasis is developing another chemically-linked conjugate of MMF and docosahexaenoic acid (CAT4001) with potential for enhanced cell targeting, efficacy, improved safety and tolerability compared to the effect of separate bioactive molecules. Specific enzymes release the two components inside the cell, where they simultaneously modulate multiple biological targets, including NRF2 and NF κ B in cells and animal models, and show promise for the treatment of neurodegenerative diseases, such as FRDA and amyotrophic lateral sclerosis (ALS). A similar technology is being developed by V ClinBio Inc. in their conjugates of MMF and eicosapentaenoic acid (VCB101, compound **5**, **Figure 3A** and VCB102) for the treatment of multiple sclerosis and psoriasis (**Table 1**).

Sulforaphane

A widely used naturally-occurring electrophilic activator of NRF2 is sulforaphane (SFN, 4-methylsulfinylbutyl isothiocyanate, compound **6**, **Figure 3A**). This compound was initially isolated by Paul Talalay and Yuesheng Zhang as the principal inducer of the NRF2-target

enzyme NQO1 from extracts of *Brassicaceae* plants²⁰⁸, and interacts with C151 of KEAP1^{14,209}. Notably, SFN goes through the blood-brain barrier⁵⁵ and has protective effects in numerous preclinical models of neurological conditions²¹⁰. In a double-blind placebo-controlled clinical trial in young men with autism spectrum disorder, orally administered capsules of SFN-rich broccoli sprout extracts have shown significant improvement in behavioral measures as assessed by Aberrant Behavior Checklist and Social Responsiveness Scale; the total scores on these scales reversed toward pretreatment levels upon cessation of treatment²¹¹. Three-day-old broccoli sprouts are rich in glucoraphanin, a precursor molecule that is hydrolyzed by the plant enzyme myrosinase to release SFN and glucose²¹². A similar β -thioglucosidase enzyme is present in the gut microbiota and therefore, the actual levels of released SFN are highly dependent on dietary habits and microbiome composition, are affected by antibiotic treatments²¹³ and display circadian rhythmicity²¹⁴. Nevertheless, either as sprout extract or highly purified SFN, more than 500 subjects have received over 25,000 doses, demonstrating a high safety profile. There are over 20 currently ongoing clinical trials.

Considering issues related to intellectual property surrounding a natural compound, the fact that SFN is unstable at room temperature, and the need to accurately control dosing, Evgen Pharma has developed a pharmaceutical form of SFN, SFX-01 (compound **7**, **Figure 3A**). SFX-01 is chemically synthesized SFN encapsulated in cyclodextrin to create a stable, solid-form pill or capsule with excellent bioavailability. SFX-01 is now being studied in clinical trials of subarachnoid hemorrhage (NCT02614742) and metastatic breast cancer (NCT02970682) (**Table 1**). Evgen also has a series of novel SFN-based analogs that are in preclinical evaluation. In addition, a number of nutraceutical companies

are producing preparations that contain NRF2 inducers, including SFN, with various degrees of standardization. One example is Prostaphane®, from the Nutrinov Laboratory, which is a stabilized free SFN, extracted from broccoli seeds with demonstrated benefits in managing biochemical recurrences after radical prostatectomy in a placebo-controlled clinical trial of prostate cancer²¹⁵. The bioavailability and potency of both SFX-01 and Prostaphane® are equivalent to those of the much less stable SFN²¹⁶. Another example is the encapsulated dietary supplement Avmacol (Nutramax Laboratories), which contains glucoraphanin from finely-milled broccoli seeds together with freeze-dried broccoli sprout powder to provide myrosinase. This supplement is currently in use in clinical trials to modulate disease symptoms and/or biomarkers related to autism spectrum disorder, schizophrenia and environmental pollution²¹⁷.

Cyanoenone triterpenoids

Accumulating evidence suggests that fumaric acid esters, SFN and other small electrophiles that bind covalently to KEAP1 may benefit from a larger scaffold structure to confer better selectivity (and likely potency) and more controllable pharmacokinetic/pharmacodynamic properties. The development of pentacyclic Michael acceptor-bearing cyanoenone triterpenoids may fulfill these requirements. Initially developed by Michael Sporn, Gordon Gribble and Tadashi Honda from the natural product oleanolic acid^{218,219}, these pentacyclic triterpenoids are the most potent NRF2 activators known to date²²⁰, are sensed by C151 in KEAP1, and are now under clinical development by Reata Pharmaceuticals and Kyowa Hakko Kirin Co., Ltd..

Two clinically advanced compounds, bardoxolone methyl (BARD) (compound **8**, **Figure 3A**) and omaveloxolone (compound **9**, **Figure 3A**), normalize metabolism, increase

mitochondrial energy production, boost cellular antioxidant capacity and lower ROS levels²²¹ (**Table 1**). In preclinical studies, BARD or analogs have been shown to improve kidney function by reducing inflammation, fibrosis and oxidative stress and increasing the filtration surface area in the glomerulus^{222,223}. With a modification of the selection criteria to exclude patients with signs of incipient heart failure (elevated B-type natriuretic peptide), BARD is now being tested in clinical trials for several rare conditions that are associated with chronic kidney disease, most of which have no approved treatments: these include Alport syndrome (NCT03019185), autosomal dominant polycystic kidney disease, IgA nephropathy, type 1 diabetes and focal segmental glomerulosclerosis (NCT03366337), in which pro-inflammatory and pro-fibrotic processes contribute to glomerulosclerosis and impaired kidney function²²⁴. BARD treatment has the potential to delay or prevent the decline in glomerular filtration rate that results in the need for dialysis or transplant in patients with Alport syndrome and other rare forms of chronic kidney disease (CKD). Thus, in a phase 2 clinical trial in patients with type 2 diabetes and CKD (NCT02316821), BARD treatment led to statistically significant increases in directly-measured glomerular filtration rate, and upon exclusion of patients at risk of fluid retention, was well tolerated. Based on these results, Kyowa Hakko Kirin has initiated a phase 3 clinical trial (NCT03550443) in diabetic patients with stage G3 or G4 CKD, with the support of the Japanese SAKIGAKE Designation system.

In addition, BARD is being studied in patients with pulmonary arterial hypertension (NCT03068130) and pulmonary arterial hypertension due to connective tissue disease with pulmonary arterial hypertension (CTD-PAH) (NCT02657356), which is a serious and progressive disease that leads to heart failure and death. The difference between CTD-PAH and idiopathic etiologies is largely attributed to the complex interplay between

inflammation, autoimmunity, and systemic vasculopathy that contribute to the pathogenesis of connective tissue disease. CTD-PAH patients have increased inflammatory processes due to upregulation of NF- κ B²²⁵, are less responsive to existing vasodilator therapies than patients with the idiopathic PAH, and have a worse prognosis²²⁶. BARD was tested in CTD-PAH patients in a phase 2 study and is currently being tested in phase 3. Omaveloxolone is currently being tested in a registrational phase 2 clinical trial for patients with FRDA (NCT02255435), a genetic neuromuscular disorder in which NRF2 is downregulated¹⁷⁶⁻¹⁷⁸. Of note, there are no currently approved therapies for the treatment of FRDA²²⁷ (**Table 1**).

Nitro fatty acids

Nitrated derivatives of fatty acids (NO₂-FA) are endogenous signaling mediators with anti-inflammatory and anti-fibrotic activities in preclinical animal models of metabolic and inflammatory disease²²⁸. The nitroalkene group confers electrophilicity to their β -carbon, promoting the rapid formation of reversible NO₂-FA Michael adducts with nucleophiles, such as cysteines, a modification termed nitroalkylation^{229,230}. It has been shown that nitro oleic acid (NO₂-OA, 9-nitro-octadec-9-enoic acid) reacts with cysteines in KEAP1, including C273 and C288, thereby activating NRF2^{231,232}. The reversibility of this reaction^{229,230,233} prevents the possibility for accumulation of stable NO₂-FA thiol adducts, which could lead to cytotoxicity. Indeed, phase 1 safety evaluation has demonstrated that the lead compound, CXA10 (10-NO₂-OA, 10-nitro-octadec-9-enoic acid, a specific regioisomer of NO₂-OA) (compound **10**, **Figure 3A**) is safe in humans at pharmacologically active doses²³⁴. CXA10 is currently being developed by Complexa Inc.

as a treatment for focal segmental glomerulosclerosis and pulmonary arterial hypertension²³⁴ (**Table 1**). The synthesis and biological evaluation of nitroalkenes derived from α -tocopherol was also recently reported²³⁵.

Hydroxylamine

A special challenge is to deliver NRF2 activators that can pass the blood-brain barrier. The route of administration (injection, oral, topical) is also crucial for specific diseases. Othera Pharmaceuticals (now acquired by Colby Pharmaceutical Co.) is developing a di-substituted hydroxylamine with antioxidant properties (OT551) for topical use that inhibits oxidative stress and disease-associated inflammation by targeting KEAP1 (compound **11**, **Figure 3A**). In preclinical studies, an ophthalmic solution of this compound protected the retinal pigment epithelium and photoreceptors from oxidative damage and inflammation, and a phase 2 trial on age-related macular degeneration has demonstrated efficacy in preventing the progression of vision loss (NCT00485394) (**Table 1**).

TFM735

A high-throughput screening reporter strategy assessing the stability of a chimeric protein comprising the NRF2 N-terminal domain fused to LacZ (NRF2d-LacZ)²³⁶ identified TFM735 as a lead compound (compound **12**, **Figure 3A**). TFM735 activates NRF2 in a C151-dependent manner, inhibits the synthesis of IL-6 and IL-17 from stimulated human peripheral blood mononuclear cells, and the progression of experimental autoimmune encephalomyelitis in mice²³⁷. This compound is currently in preclinical development by Mochida Pharmaceuticals for the treatment of multiple sclerosis (**Table 1**).

NRF2/KEAP1 protein-protein interaction inhibitors

Recent attention has focused on the development of non-electrophilic non-covalent compounds that interfere with the direct protein-protein interaction (PPI) between KEAP1 and NRF2²³⁸, or the PPI between KEAP1 and Cul3²³⁹. By interfering directly with the interaction between KEAP1 and NRF2 or Cul3, the compounds do not require a covalent binding component to their mode of action. There are potential advantages to this approach, including the scope to explore new chemotypes of NRF2 inducers, different pharmacodynamics due to a different mode of interaction with KEAP1 and different off-target effect profiles due to a cysteine-independent binding mechanism. There is evidence that electrophilic and non-electrophilic (PPI) KEAP1 inhibitors differ in their spectrum of biological effects, an example being the ability of non-electrophiles to induce mitophagy in contrast to electrophiles such as SFN and DMF; this points towards potential differences in pharmacological activity and therapeutic utility between the two compound classes²⁴⁰.

The design of PPI inhibitors has been guided by the availability of the crystal structure of the Kelch domain of KEAP1²⁴¹⁻²⁴³ (**Figure 3B**). Several types of PPI inhibitors have been reported; the major chemical classes developed with input from the pharmaceutical industry are shown in **Figure 3B**. The naphthalene *bis*-sulfonamide compounds originated from a high-throughput screening campaign at Biogen²⁴⁴ and subsequently elaborated by Jiang *et al.*²⁴⁵ at China Pharmaceutical University to provide compound **13** (CPUY192018) and analogues. This *bis*-carboxylic acid compound is a high-affinity ligand for KEAP1, which induced the expression of ARE-dependent genes at low micromolar concentrations. Astex and GlaxoSmithKline also identified sulfonamide-containing lead compounds from an x-ray crystallography fragment-based screening program. The lead compounds in this case are exemplified by compound **14**, a low

nanomolar inhibitor of the KEAP1-NRF2 PPI that increased the expression of the NRF2-target gene *NQO1* in treated COPD patient-derived epithelial cells. The compound was capable of reducing lung inflammation in rats after *i.v.* administration and effectively suppressed ozone-induced accumulation of leukocytes in bronchoalveolar fluid and restored GSH concentrations²⁴⁶. Compounds from this series have shown efficacy in several animal models of oxidative stress that simulate features of pulmonary disease and are currently being developed for clinical evaluation. Two further classes of mono-acidic PPI inhibitors have been developed which are not sulfonamides. The first class, developed at Rutgers by Hu *et al.*²⁴⁷ and elaborated by Evotec and UCB Pharma²⁴⁸, were the tetrahydroisoquinolines, *e.g.* compound **15**, which were low micromolar inhibitors of the KEAP1-NRF2 PPI. The second class was developed by Toray Industries and RIKEN and incorporated an oxadiazole motif; compound **16** is an inhibitor of the PPI with binding activity in the micromolar range²⁴⁹. The highlighted compounds or their analogues have been co-crystallised with the KEAP1 Kelch domain; **Figure 3B** illustrates the occupancy of the binding site sub-pockets that compounds **13-16** achieve and provide insights into how these ligands may be refined in the future.

New PPI inhibitors are also being developed by C4X Discovery using a combination of computational chemistry, ligand nuclear magnetic resonance (NMR) spectroscopy and protein crystallography approaches and by Keapstone, using a structure-based drug design approach. Academic groups are also contributing to the pool of non-electrophilic NRF2 inducers^{250,251}.

A significant challenge remains in making PPI inhibitors that possess suitable drug metabolism and pharmacokinetic properties for use in both peripheral and central nervous system applications, the latter being an area in which KEAP1 inducers with a strong safety

profile are required for chronic administration for conditions such as AD and PD. At this time, the potential advantage of improved target selectivity of the non-electrophilic PPI inhibitors is offset by the relatively high molecular weights of the current compounds, a requirement for blocking the large KEAP1-NRF2 interface, and the need for polar functional groups to confer tight KEAP1-binding affinity. Thus, many of the prototype compounds that were identified by *in vitro* studies, at present exhibit poor absorption, distribution, metabolism and excretion properties. For instance, the naphthalene *bis*-sulfonamide **13** protects against dextran sodium sulfate (DSS)-induced colitis in mice, activates NQO1 expression in cultured NCM460 cells in the low micromolar range, and binds KEAP1 *in vitro*, demonstrating the offset between protein binding and biological activities²⁵².

The majority of the *in vivo* evaluations of PPI inhibitors have focused on peripheral inflammatory conditions. For example, the compound NK-252 (an analogue of **16**) was identified by Toray Industries as a relatively weak KEAP1 inhibitor, but had a protective effect against H₂O₂-induced cytotoxicity and reduced the fibrosis score in rats fed with a choline-deficient L-amino acid-defined diet²⁵³, a model system for NASH.

Targeting NRF2 with KEAP1-independent drugs

Increasing evidence indicates that NRF2 exhibits multiple layers of regulation in addition to that exerted by KEAP1, such as transcriptional¹⁹⁶, epigenetic^{254,255}, covalent protein modification^{256,257}, proteasome degradation in KEAP1-independent manner²⁵⁸⁻²⁶¹, and regulation of NRF2-dimerizing partners that target binding to ARE sequences^{262,263}. At the level of pharmacological development, a KEAP1-independent mechanism of ARE-mediated gene regulation involves modulation of the transcriptional repressor BACH1 (i.e.,

broad complex, tramtrack and bric à brac and cap'n'collar homology 1), which inhibits the transactivation of a subset of NRF2-regulated genes and more specifically HMOX1²⁸. VTV Therapeutics is now analyzing if this subset is sufficient to elicit therapeutic benefits. They have developed a novel class of non-electrophilic small molecules that inhibit BACH1 binding to some ARE-driven genes independently of KEAP1²⁶⁴.

Challenges and considerations

Pharmacodynamics assessment

Considering that NRF2 has a very short half-life, even after drug-induced stabilization, the most appropriate dosing regimen for a therapeutic benefit needs to be inferred by indirect indicators of its activation in the diseased organs. One possibility is to analyze drug distribution and NRF2 gene expression signatures in accessible cells or tissues (such as peripheral blood mononuclear cells, nasal lavage fluid cells, exfoliated bladder cells, buccal cells and skin) with the hope that the transcriptomic signature of NRF2 in these cells reflects local engagement in other tissues (such as the lung, liver, and brain)²⁶⁵⁻²⁶⁷. In the case of communities unavoidably exposed to harmful environmental chemicals, the urinary levels of their corresponding metabolites have been used as biomarkers of pharmacodynamic action mediated through NRF2^{268,269}.

Activation of the NRF2 pathway is characterized by a time-, tissue-, dose-, onset-, and duration-dependent differential gene expression. **Table 2** shows the half-lives of a representative subset of NRF2-regulated gene products. As many of these proteins have a relatively long half-life, their biochemical activities are expected to last much longer than the time interval over which NRF2 is stabilized or the presence of its pharmacological activator, the latter of which is often cleared within several hours. As such, the

pharmacodynamics of NRF2-activating molecules are observed over much longer periods of time than the levels of NRF2, and do not correspond to the plasma drug concentrations. Thus, the NRF2 activator TBE-31, a tricyclic cyanoenone closely related to the triterpenoids described above, has a half-life of 10 h in murine skin and plasma^{51,270}, whereas NRF2-dependent induction of NQO1 protein is still evident in the skin of the animals three days after the last dose of topically-applied drug⁵¹. Based on the fact that NRF2 activators lead to the “stimulation of the production or inhibition of factors which control the measured effect”, an “indirect pharmacodynamic response” model^{271,272} may be a more logical approach for testing the NRF2 activator pharmacodynamics towards clinical applications.

The concentration and distribution of NRF2-activating drugs in the relevant tissue, as well as their solubility, cell permeability, metabolic stability, and protein binding, will also play a major role in the extent of NRF2 activation, ultimately leading to the desired long-lasting pharmacodynamic effects. Therefore, optimization of clinically meaningful drug exposure requires an analysis of dose-dependent gene regulation by a putative NRF2 activator. Furthermore, NRF2 target engagement studies must consider patient age and performance status, as the ability of drugs to activate NRF2 or promote an “adaptive response” appears to be reduced in older and less healthy subjects. This was recently seen in a clinical trial of DMF in patients with relapsing-remitting multiple sclerosis, where it was found that the degree of induction of the NRF2 transcriptional target *NQO1* negatively correlated with patient age, and that patients with higher NQO1 protein levels at 4-6 weeks after the start of therapy were more likely to show no evidence of disease activity during the following year²⁶⁷.

Drug selectivity

The identification of highly reactive cysteines in KEAP1 was crucial for the understanding of the mechanism by which electrophilic compounds interact covalently with this ubiquitin ligase substrate adaptor. Structural information suggests that certain drugs acting on C151 may disrupt the interaction with Cullin-3 (Cul3)/Rbx1^{273,274}, thus leaving the KEAP1 pool saturated with bound NRF2 and allowing newly synthesized NRF2 to evade degradation: this was demonstrated using the model electrophile *N*-iodoacetyl-*N*-biotinylhexylenediamine²⁷⁵. Other compounds may modify KEAP1 in a way that it is no longer capable of interacting with NRF2 at both the high- and low-affinity sites within the transcription factor. Notably, some electrophilic NRF2 activators, such as the cyclic cyanoenones bind to thiols covalently but do not form irreversible adducts^{276,277}. Consequently, they combine the desirable features of both irreversible covalent drugs (i.e., high potency and sustained target engagement), as well as that of reversible non-covalent drugs (i.e., absence of permanent modification and therefore possible destruction or immunogenicity of their protein targets)²⁷⁸. A potential drawback of such molecules is that they might react with redox-sensitive cysteines in proteins other than KEAP1, hence compromising their function. The issue of specificity of thiol-reactive molecules indicates that the window that allows for therapeutically relevant KEAP1 modification in the absence of thiol modifications within other proteins needs to be determined for each compound (**Figure 1B**). Such a hierarchical presentation of target cysteines within KEAP1 as well as other thiol-containing non-target proteins has been termed the “cysteine code”²⁷⁹ and is influenced by both the chemistry and dose of inducer²⁸⁰.

A significant potential advantage of KEAP1-NRF2 PPI inhibitors is improved target selectivity. However, off-target effects cannot be completely excluded *a priori* for any

small molecule. In addition, KEAP1 targets proteins other than NRF2²⁸¹ for ubiquitination, and these might also be affected. One of the few types of PPI inhibitors for which off-target selectivity has been evaluated are the potent mono acidic sulfonamides, for example, compound **10** appeared to be relatively selective for KEAP1, showing modest activity in a GlaxoSmithKline enhanced cross-screen panel of *in vitro* assays to identify potential off-target effects²⁴⁶.

Animal models

A problem that precludes successful drug development for most chronic diseases is the incomplete reproducibility of human pathologies in animal models²⁸². This issue may be associated with the lack of a very important hallmark of human degenerative diseases, namely the progressive loss of homeostatic functions associated with NRF2. In fact, although much work still needs to be done in humans, evidence from animal studies indicates that NRF2 activity declines with aging and that the pharmacological or genetic up-regulation of NRF2 increases life-span and improves health-span^{283,284}. The lack of an “NRF2 variable” in animal models of chronic diseases is well exemplified in murine models of AD. The current models are based on the assumption that expression of toxic APP or TAU mutant proteins in an otherwise healthy animal background replicates the human pathology. While these models provide valuable information about the onset and progression of the proteinopathy, they have systematically failed to translate therapeutic success to humans. The reasons for this failure may be related to the fact that these models do not consider the disease-related decline of homeostatic functions such as those related to NRF2. More sophisticated preclinical models will require a reverse translational approach in which not only the anatomopathological features of a specific disease are replicated in

animals, but also the compromised redox, inflammatory or protein homeostasis. Thus, the NRF2-knockout mouse shares many alterations common to AD patients or even aged individuals¹⁵⁶. Recent studies have demonstrated that compared to wild-type animals, NRF2-knockout mice fed high-fat diet display greater neurovascular dysfunction, blood-brain barrier disruption, neuroinflammation, amyloidogenic gene expression, and cognitive decline, mimicking many of the phenotypic changes that occur with aging²⁸⁵. The introduction of low NRF2 expression as a variable to reduce homeostatic responses may be useful to improve current models of chronic diseases towards better therapeutic outcomes. Considering the slow decline of NRF2 activity with aging, the heterozygous *Nrf2*^{+/-} mouse might be a suitable model to replicate both reduced basal expression and pharmacological upregulation.

Cancer risk

From a pharmacological point of view, it is necessary to determine how significant is the risk posed by increasing NRF2 levels beyond a safe threshold of causing cancer (**Figure 4**). The impact of somatic mutations that promote NRF2 stability in cancer cells is markedly different to that caused by pharmacologic activation of NRF2. Thus, somatic mutations in *KEAP1* and *NFE2L2* result in high, unrestrained NRF2 activity which is entirely distinct from the pulsatile activation caused by pharmacologic administration of NRF2 activators. Kinetic features such as amplitude and areas under the curve, reflecting intensity and duration of NRF2 signaling, are vastly different in settings of activation by genetic versus pharmacological means^{202,286}. Moreover, gene loss or inactivating mutations in *KEAP1* do not appear to have the same effect as pharmacological inhibition of this protein. Thus, KEAP1 loss increased the levels of NRF2 but also several oncogenic targets of this E3

ligase adapter, including inhibitor of NF- κ B kinase subunit beta (IKK β) and B-cell lymphoma 2 (BCL2) that might be at least partially responsible for malignant transformation. Accordingly, in a panel of cell lines with functional KEAP1, a well-established NRF2 activator, the BARD analog RTA-405, increased NRF2 but not IKK β or BCL2 levels, and did not confer a growth or survival advantage to tumor cells²⁸⁷.

While at the present time a cancer risk from NRF2 activators cannot be dismissed, it is encouraging to see that a meta-analysis of a phase 3 trial of DMF has shown no difference in the cancer rate between the placebo and DMF-treated groups²⁸⁸. It is also important to note that some NRF2-activating drugs may reduce this risk if they have additional targets with antitumor effects. Thus, DMF was recently found to inhibit GAPDH²⁸⁹, and it is possible that such inhibition may lead to energetic crisis in highly glycolytic *KRAS* or *BRAF* mutant cancer cells thus preventing tumor development as shown for vitamin C²⁹⁰.

Outlook

A hallmark of many chronic diseases is the loss of homeostatic responses such as redox signaling, metabolic flexibility, controlled inflammation and proteostasis. The multifactorial nature of these complex diseases could be targeted with one single hit at the transcription factor NRF2 as its activation elicits beneficial comprehensive, “multi-target” and long-lasting cytoprotective effects. Slowly but steadily biopharmaceutical companies are developing drugs that target KEAP1, the main regulator of NRF2 even though the peculiarity of the KEAP1/NRF2 system makes this approach particularly challenging for monitoring target engagement and off-target effects. In addition, the concern about an increased cancer risk needs to be definitively excluded. Over the next few years, the

ongoing clinical trials will undoubtedly provide significant advances in answering these questions.

Box 1. Emergence of NRF2 inhibitors for cancer treatment

Somatic mutations in the KEAP1/NRF2 axis have been found in several tumor types¹⁹³, suggesting a potential benefit of NRF2 inhibitors in cancer treatment. Following ligand-mediated receptor activation and binding to the glucocorticoid response element, agonists of the glucocorticoid receptor, such as dexamethasone, inhibit the transcriptional activity of NRF2, at least on the *GSTA2* gene²⁹¹. Agonists of the nuclear receptors retinoic acid receptor alpha (RAR α) and retinoid X receptor alpha (RXR α), including all-*trans*-retinoic acid, also inhibit the transcriptional activity of NRF2 in an ARE-reporter cell line and in the small intestine of mice fed a vitamin A-deficient diet^{292,293}. It is likely that these mechanisms of regulation through nuclear receptors are not specific for NRF2, though RXR α has been reported to interact directly with NRF2²⁹³.

Among pharmacological agents, brusatol, a quassinoid extracted from *Brucea javanica*, attracted initial attention as it reduced the expression of an ARE-luciferase reporter and sensitized a broad spectrum of cancer cells to cisplatin in culture and in xenografts²⁹⁴. However, it is now recognized that brusatol is a general inhibitor of protein synthesis, thus leading to the preferential inhibition of short-lived proteins, including but not limited to NRF2^{295,296}. A similar concern was raised after the identification of the febrifugine derivative halofuginone in a high-throughput inhibitor screen in chemo- and radio-resistant cancer cells²⁹⁷. Halofuginone induced a cellular amino acid starvation response that repressed global protein synthesis and rapidly depleted NRF2 and other

proteins. The flavonoid luteolin was reported to accelerate the turnover of NRF2 mRNA and thus elicit a strong reduction of NRF2 protein and mRNA levels, and sensitize cells to anticancer drugs²⁹⁸. However, subsequent studies provided conflicting results as they report that luteolin is an NRF2 activator^{299,300}. Another flavonoid, wogonin, has also yielded conflicting results^{301,302}. The coffee alkaloid trigonelline inhibited the nuclear accumulation of NRF2 protein³⁰³ and reduced NRF2-dependent proteasome activity in several pancreatic cancer cell lines³⁰⁴. Nevertheless, these effects need to be addressed in a wider range of cell types before being taken to preclinical studies.

Targeting the interaction between NRF2 and sMAF proteins might be a promising strategy. Thus, a quantitative high-throughput screen identified a thiazole-indoline compound as an NRF2 inhibitor, and medicinal chemistry optimization led to compound ML385 that was found to bind the C-terminal domain of NRF2 and interfere with the formation of the NRF2/sMAF protein heterodimer that is required for activation of ARE-driven gene expression³⁰⁵. Further work is needed to determine if ML385 is selective for NRF2/sMAF interaction or also affects other basic-region leucine zipper transcription factors. Moreover, this compound elicits a reduction of NRF2 protein levels, and therefore additional mechanisms of NRF2 regulation may operate. Other NRF2 inhibitors with unknown mechanism of action include malabricone A³⁰⁶, ochratoxin A³⁰⁷ and AEM1³⁰⁸. However, these compounds do not appear to be specific for NRF2 inhibition either.

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Glossary terms

Antioxidant Response Element (ARE)

Specific DNA sequences, which are present in the promoter regions of NRF2-target genes. The ARE was discovered before NRF2, and its name originates from the fact that it was first identified in the promoter regions of genes that are induced by phenolic antioxidants.

Autophagosome

A spherical double-layer membrane structure within the cell. The autophagosome is the key structure in macroautophagy, a type of intracellular degradation process.

Clara cells

Dome-shaped cells found in the bronchioles of the lungs.

Chronic Obstructive Pulmonary Disease

Progressive lung disease including emphysema, chronic bronchitis, and refractory (non-reversible) asthma.

Emphysema

Progressive lung disease that causes shortness of breath due to over-inflation of the alveoli.

Alport Syndrome

Genetic disorder of the glomerular basement membrane, part of the glomerular filtration unit, characterized by kidney disease, hearing loss, and eye abnormalities.

Gluconeogenesis

Metabolic process that generates glucose from non-carbohydrate carbon substrates, such as lactate, glycerol, and glucogenic amino acids.

Interferonopathies

Mendelian disorders associated with an upregulation of interferon.

Proteostasis

Protein homeostasis.

Redox stress

An imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control, and/or molecular damage.

Abbreviations

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ARE, antioxidant response element; BACH1, broad complex, tramtrack and bric à brac and cap'n'collar homology 1; BARD, bardoxolone methyl; BTB, Bric-à-Brac domain of KEAP1; CKD, chronic kidney

disease; COPD, chronic obstructive pulmonary disease; CTD, connective tissue disease; DMF, dimethyl fumarate; FA, fatty acid; FRDA, Friedreich's ataxia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, reduced form of glutathione; GST, glutathione *S*-transferase; HMOX1, heme oxygenase 1; IVR, intervening region of KEAP1; KEAP1, Kelch-like ECH-associated protein 1; LC3, microtubule Associated Protein 1 Light Chain 3 Alpha; MMF, monomethyl fumarate; mtDNA, mitochondrial DNA; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NF- κ B, nuclear factor-kappaB; NO₂-FA, Nitrated derivatives of fatty acids; NQO1, NAD(P)H:quinone oxidoreductase 1; NRF2, Nuclear factor (erythroid-derived 2) p45-related factor 2; PAH, pulmonary arterial hypertension; PD, Parkinson's disease; PPI, protein-protein interaction; ROS, reactive oxygen species; SFN, sulforaphane; sMAF, small musculoaponeurotic fibrosarcoma oncogene homolog; SQSTM1, sequestosome 1; STING, stimulator of interferon genes; UBA, ubiquitin association domain of SQSTM1.

References

- 1   Moi, P., Chan, K., Asunis, I., Cao, A. & Kan, Y. W. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* **91**, 9926-9930 (1994).
Identification of NRF2 as a member of the human cap'n'collar (CNC) basic-region leucine zipper transcription factor family.
- 2   Itoh, K. *et al.* An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* **236**, 313-322 (1997).
Identification of NRF2 as the transcription factor that mediates the expression of cytoprotective genes through antioxidant response elements.
- 3   Chan, K., Lu, R., Chang, J. C. & Kan, Y. W. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. *Proc Natl Acad Sci U S A* **93**, 13943-13948 (1996).
- 4   Hayes, J. D. & Dinkova-Kostova, A. T. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem Sci* **39**, 199-218, doi:10.1016/j.tibs.2014.02.002 (2014).

- 5 Itoh, K. *et al.* Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* **13**, 76-86 (1999).

Describes the identification of KEAP1 as the main negative regulator of NRF2.

- 6 Zhang, D. D., Lo, S. C., Cross, J. V., Templeton, D. J. & Hannink, M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* **24**, 10941-10953, doi:10.1128/MCB.24.24.10941-10953.2004 (2004).

Describes that KEAP1 functions as a substrate adaptor protein for Cul3-dependent ubiquitination of NRF2.

- 7 Cullinan, S. B., Gordan, J. D., Jin, J., Harper, J. W. & Diehl, J. A. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. *Mol Cell Biol* **24**, 8477-8486, doi:10.1128/MCB.24.19.8477-8486.2004 (2004).

Describes that KEAP1 functions as a substrate adaptor protein for Cul3-dependent ubiquitination of NRF2.

- 8 Kobayashi, A. *et al.* Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* **24**, 7130-7139, doi:10.1128/MCB.24.16.7130-7139.2004 (2004).

Describes that KEAP1 functions as a substrate adaptor protein for Cul3-dependent ubiquitination of NRF2.

- 9 Cuadrado, A. *et al.* Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine Approach. *Pharmacol Rev* **70**, 348-383, doi:10.1124/pr.117.014753 (2018).

The first attempt to address the role of NRF2 in human chronic disease from a systems medicine perspective; includes a map of disease mechanisms underlined by NRF2 alterations, the NRF2-diseasome, and a connectivity map of NRF2 with other physically or functionally associated proteins, the NRF2-interactome.

- 10 Yamamoto, M., Kensler, T. W. & Motohashi, H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. *Physiol Rev* **98**, 1169-1203, doi:10.1152/physrev.00023.2017 (2018).

- 11 Liby, K. T. & Sporn, M. B. Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. *Pharmacol Rev* **64**, 972-1003, doi:10.1124/pr.111.004846 (2012).

Comprehensive review of the cyanoenone triterpenoids and their broad applicability for prevention and treatment of chronic disease.

- 12 Rojo de la Vega, M., Chapman, E. & Zhang, D. D. NRF2 and the Hallmarks of Cancer. *Cancer Cell* **34**, 21-43, doi:10.1016/j.ccell.2018.03.022 (2018).

- 13 Ogura, T. *et al.* Keap1 is a forked-stem dimer structure with two large spheres enclosing the intervening, double glycine repeat, and C-terminal domains. *Proc Natl Acad Sci U S A* **107**, 2842-2847, doi:10.1073/pnas.0914036107 (2010).

- 14 McMahon, M., Lamont, D. J., Beattie, K. A. & Hayes, J. D. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. *Proc Natl Acad Sci U S A* **107**, 18838-18843, doi:10.1073/pnas.1007387107 (2010).

Describes that KEAP1 has distinct cysteine sensors.

- 15 Takaya, K. *et al.* Validation of the multiple sensor mechanism of the Keap1-Nrf2 system. *Free Radic Biol Med* **53**, 817-827, doi:10.1016/j.freeradbiomed.2012.06.023 (2012).

- 16 Saito, R. *et al.* Characterizations of Three Major Cysteine Sensors of Keap1 in Stress Response. *Mol Cell Biol* **36**, 271-284, doi:10.1128/MCB.00868-15 (2015).

- 17 Sihvola, V. & Levonen, A. L. Keap1 as the redox sensor of the antioxidant response. *Arch Biochem Biophys* **617**, 94-100, doi:10.1016/j.abb.2016.10.010 (2017).

18 Dinkova-Kostova, A. T., Kostov, R. V. & Canning, P. Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. *Arch Biochem Biophys* **617**, 84-93, doi:10.1016/j.abb.2016.08.005 (2017).

19 Dinkova-Kostova, A. T. *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* **99**, 11908-11913, doi:10.1073/pnas.172398899 (2002).

Describes the finding that cysteine sensors in KEAP1 regulate NRF2-dependent gene expression.

20 Kumar, H., Kim, I. S., More, S. V., Kim, B. W. & Choi, D. K. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. *Nat Prod Rep* **31**, 109-139, doi:10.1039/c3np70065h (2014).

21 Crunkhorn, S. Deal watch: Abbott boosts investment in NRF2 activators for reducing oxidative stress. *Nature reviews. Drug discovery* **11**, 96, doi:10.1038/nrd3655 (2012).

22 Sies, H., Berndt, C. & Jones, D. P. Oxidative Stress. *Annu Rev Biochem* **86**, 715-748, doi:10.1146/annurev-biochem-061516-045037 (2017).

23 Thimmulappa, R. K. *et al.* Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* **62**, 5196-5203 (2002).

One of the earliest microarray analyses of the NRF2-dependent effects of sulforaphane in mice; identifies the importance of NRF2 in regulating the levels of NADPH.

24 Lee, J. M., Calkins, M. J., Chan, K., Kan, Y. W. & Johnson, J. A. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem* **278**, 12029-12038, doi:10.1074/jbc.M211558200 (2003).

25 Wu, K. C., Cui, J. Y. & Klaassen, C. D. Beneficial role of nrf2 in regulating NADPH generation and consumption. *Toxicol Sci* **123**, 590-600, doi:10.1093/toxsci/kfr183 (2011).

26 Mitsuishi, Y. *et al.* Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* **22**, 66-79, doi:10.1016/j.ccr.2012.05.016 (2012).

Describes the role of NRF2 in regulating the pentose phosphate pathway in cancer cells; provides the basis to understand how NRF2 participates in the metabolic alterations of cancer cells.

27 Tanito, M., Agbaga, M. P. & Anderson, R. E. Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro. *Free Radic Biol Med* **42**, 1838-1850, doi:10.1016/j.freeradbiomed.2007.03.018 (2007).

28 MacLeod, A. K. *et al.* Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis* **30**, 1571-1580, doi:10.1093/carcin/bgp176 (2009).

29 Agyeman, A. S. *et al.* Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. *Breast Cancer Res Treat*, doi:10.1007/s10549-011-1536-9 (2011).

30 Sedlak, T. W. *et al.* Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci U S A* **106**, 5171-5176, doi:10.1073/pnas.0813132106 (2009).

31 Holmstrom, K. M. *et al.* Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. *Biology open* **2**, 761-770, doi:10.1242/bio.20134853 (2013).

One of the first studies that describes the role of NRF2 in regulating mitochondrial function.

- 32 Hu, Q. *et al.* The mitochondrially targeted antioxidant MitoQ protects the intestinal barrier by ameliorating mitochondrial DNA damage via the Nrf2/ARE signaling pathway. *Cell Death Dis* **9**, 403, doi:10.1038/s41419-018-0436-x (2018).
- 33 Komatsu, M. *et al.* The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* **12**, 213-223, doi:10.1038/ncb2021 (2010).

One of the first studies that describes the role of NRF2 in regulating the expression of p62.

- 34 Jain, A. *et al.* p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* **285**, 22576-22591, doi:10.1074/jbc.M110.118976 (2010).

One of the first studies that describes the role of NRF2 in regulating the expression of p62.

- 35 Lau, A. *et al.* A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. *Mol Cell Biol* **30**, 3275-3285, doi:10.1128/MCB.00248-10 (2010).

One of the first studies that describes the role of NRF2 in regulating the expression of p62.

- 36 Lee, Y. *et al.* Keap1/Cullin3 Modulates p62/SQSTM1 Activity via UBA Domain Ubiquitination. *Cell reports* **19**, 188-202, doi:10.1016/j.celrep.2017.03.030 (2017).
- 37 Ichimura, Y. *et al.* Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol Cell* **51**, 618-631, doi:10.1016/j.molcel.2013.08.003 (2013).
- 38 Taguchi, K. *et al.* Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc Natl Acad Sci U S A* **109**, 13561-13566, doi:10.1073/pnas.1121572109 (2012).
- 39 Pajares, M. *et al.* Transcription factor NFE2L2/NRF2 modulates chaperone-mediated autophagy through the regulation of LAMP2A. *Autophagy*, doi:10.1080/15548627.2018.1474992 (2018).
- 40 Pajares, M. *et al.* Transcription factor NFE2L2/NRF2 is a regulator of macroautophagy genes. *Autophagy* **12**, 1902-1916, doi:10.1080/15548627.2016.1208889 (2016).

One of the first studies that describes the role of NRF2 in regulating autophagy.

- 41 Dayalan Naidu, S. *et al.* Transcription factors NRF2 and HSF1 have opposing functions in autophagy. *Sci Rep* **7**, 11023, doi:10.1038/s41598-017-11262-5 (2017).
- 42 Cullinan, S. B. *et al.* Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* **23**, 7198-7209 (2003).

Links NRF2 with endoplasmic reticulum (ER) stress.

- 43 Pajares, M., Cuadrado, A. & Rojo, A. I. Modulation of proteostasis by transcription factor NRF2 and impact in neurodegenerative diseases. *Redox biology* **11**, 543-553, doi:10.1016/j.redox.2017.01.006 (2017).
- 44 Kwak, M. K., Wakabayashi, N., Greenlaw, J. L., Yamamoto, M. & Kensler, T. W. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol* **23**, 8786-8794 (2003).
- 45 Kwak, M. K. & Kensler, T. W. Induction of 26S proteasome subunit PSMB5 by the bifunctional inducer 3-methylcholanthrene through the Nrf2-ARE, but not the AhR/Arnt-XRE, pathway. *Biochem Biophys Res Commun* **345**, 1350-1357, doi:10.1016/j.bbrc.2006.05.043 (2006).
- 46 Bolanos, J. P. Bioenergetics and redox adaptations of astrocytes to neuronal activity. *J Neurochem* **139 Suppl 2**, 115-125, doi:10.1111/jnc.13486 (2016).

- 47 Shih, A. Y. *et al.* Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potentially protects neurons from oxidative stress. *J Neurosci* **23**, 3394-3406 (2003).
- 48 Thimmulappa, R. K. *et al.* Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. *J Clin Invest* **116**, 984-995, doi:10.1172/JCI25790 (2006).

One of the first studies showing the anti-inflammatory role of NRF2.

- 49 Braun, S. *et al.* Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. *Mol Cell Biol* **22**, 5492-5505 (2002).
- 50 Long, M. *et al.* An Essential Role of NRF2 in Diabetic Wound Healing. *Diabetes* **65**, 780-793, doi:10.2337/db15-0564 (2016).
- 51 Knatko, E. V. *et al.* Nrf2 Activation Protects against Solar-Simulated Ultraviolet Radiation in Mice and Humans. *Cancer Prev Res (Phila)* **8**, 475-486, doi:10.1158/1940-6207.CAPR-14-0362 (2015).
- 52 Kobayashi, E. H. *et al.* Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nature communications* **7**, 11624, doi:10.1038/ncomms11624 (2016).

Reports a novel mechanism of NRF2 in repressing the expression of pro-inflammatory genes.

- 53 Quinti, L. *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* **114**, E4676-E4685, doi:10.1073/pnas.1614943114 (2017).
- 54 Innamorato, N. G. *et al.* The transcription factor Nrf2 is a therapeutic target against brain inflammation. *J Immunol* **181**, 680-689 (2008).
- 55 Jazwa, A. *et al.* Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism. *Antioxid Redox Signal* **14**, 2347-2360, doi:10.1089/ars.2010.3731 (2011).
- 56 Cuadrado, A., Kugler, S. & Lastres-Becker, I. Pharmacological targeting of GSK-3 and NRF2 provides neuroprotection in a preclinical model of tauopathy. *Redox biology* **14**, 522-534, doi:10.1016/j.redox.2017.10.010 (2018).
- 57 Saw, C. L. *et al.* Impact of Nrf2 on UVB-induced skin inflammation/photoprotection and photoprotective effect of sulforaphane. *Mol Carcinog* **50**, 479-486, doi:10.1002/mc.20725 (2011).
- 58 Lin, W. *et al.* Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. *Biochem Pharmacol* **76**, 967-973, doi:10.1016/j.bcp.2008.07.036 (2008).
- 59 Arisawa, T. *et al.* Nrf2 gene promoter polymorphism is associated with ulcerative colitis in a Japanese population. *Hepatogastroenterology* **55**, 394-397 (2008).
- 60 Arisawa, T. *et al.* The relationship between Helicobacter pylori infection and promoter polymorphism of the Nrf2 gene in chronic gastritis. *Int J Mol Med* **19**, 143-148 (2007).

One of the first studies to show associations between polymorphisms in the NFE2L2 gene and human disease.

- 61 Wenzel, P., Kossmann, S., Munzel, T. & Daiber, A. Redox regulation of cardiovascular inflammation - Immunomodulatory function of mitochondrial and Nox-derived reactive oxygen and nitrogen species. *Free Radic Biol Med*, doi:10.1016/j.freeradbiomed.2017.01.027 (2017).

- 62 Liu, X. *et al.* Dimethyl fumarate ameliorates dextran sulfate sodium-induced murine experimental colitis by activating Nrf2 and suppressing NLRP3 inflammasome activation. *Biochem Pharmacol* **112**, 37-49, doi:10.1016/j.bcp.2016.05.002 (2016).
- 63 Kovac, S. *et al.* Nrf2 regulates ROS production by mitochondria and NADPH oxidase. *Biochim Biophys Acta* **1850**, 794-801, doi:10.1016/j.bbagen.2014.11.021 (2014).
- 64 Rushworth, S. A. *et al.* The high Nrf2 expression in human acute myeloid leukemia is driven by NF-kappaB and underlies its chemo-resistance. *Blood* **120**, 5188-5198, doi:10.1182/blood-2012-04-422121 (2012).
- 65 Cuadrado, A., Martin-Moldes, Z., Ye, J. & Lastres-Becker, I. Transcription factors NRF2 and NF-kappaB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. *J Biol Chem* **289**, 15244-15258, doi:10.1074/jbc.M113.540633 (2014).
- 66 Kim, S. W., Lee, H. K., Shin, J. H. & Lee, J. K. Up-down regulation of HO-1 and iNOS gene expressions by ethyl pyruvate via recruiting p300 to Nrf2 and depriving it from p65. *Free Radic Biol Med* **65**, 468-476, doi:10.1016/j.freeradbiomed.2013.07.028 (2013).
- 67 Lee, D. F. *et al.* KEAP1 E3 ligase-mediated downregulation of NF-kappaB signaling by targeting IKKbeta. *Mol Cell* **36**, 131-140, doi:10.1016/j.molcel.2009.07.025 (2009).
- 68 Kim, J. E. *et al.* Suppression of NF-kappaB signaling by KEAP1 regulation of IKKbeta activity through autophagic degradation and inhibition of phosphorylation. *Cell Signal* **22**, 1645-1654, doi:10.1016/j.cellsig.2010.06.004 (2010).
- 69 Ishii, T. & Mann, G. E. Redox status in mammalian cells and stem cells during culture in vitro: critical roles of Nrf2 and cystine transporter activity in the maintenance of redox balance. *Redox Biol* **2**, 786-794, doi:10.1016/j.redox.2014.04.008 (2014).
- 70 Harvey, C. J. *et al.* Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. *Sci Transl Med* **3**, 78ra32, doi:10.1126/scitranslmed.3002042 (2011).

One of the first studies to show beneficial effects of NRF2 targeting in human lung disease.

- 71 Mills, E. L. *et al.* Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* **556**, 113-117, doi:10.1038/nature25986 (2018).

Describes the mechanism by which an endogenous electrophile connects immunometabolism with upregulation of NRF2 and suppression of inflammation in macrophages.

- 72 Bambouskova, M. *et al.* Electrophilic properties of itaconate and derivatives regulate the IkappaBzeta-ATF3 inflammatory axis. *Nature* **556**, 501-504, doi:10.1038/s41586-018-0052-z (2018).
- 73 Olgarnier, D. *et al.* Activation of Nrf2 Signaling Augments Vesicular Stomatitis Virus Oncolysis via Autophagy-Driven Suppression of Antiviral Immunity. *Mol Ther* **25**, 1900-1916, doi:10.1016/j.ymthe.2017.04.022 (2017).

Describes that NRF2 suppresses antiviral immunity, which can be exploited therapeutically in oncolytic infection.

- 74 Olgarnier, D. *et al.* Nrf2 negatively regulates STING indicating a link between antiviral sensing and metabolic reprogramming. *Nature communications* **9**, 3506, doi:10.1038/s41467-018-05861-7 (2018).
- 75 Cho, H. Y. Genomic structure and variation of nuclear factor (erythroid-derived 2)-like 2. *Oxid Med Cell Longev* **2013**, 286524, doi:10.1155/2013/286524 (2013).

Analysis of polymorphisms in NFE2L2, the gene encoding NRF2, that are associated with human disease.

- 76 von Otter, M. *et al.* Nrf2-encoding NFE2L2 haplotypes influence disease progression but not risk in Alzheimer's disease and age-related cataract. *Mech Ageing Dev* **131**, 105-110, doi:10.1016/j.mad.2009.12.007 (2010).

- 77 von Otter, M. *et al.* Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease. *BMC Med Genet* **11**, 36, doi:10.1186/1471-2350-11-36 (2010).
- 78 Alam, J. *et al.* Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. *J Biol Chem* **275**, 27694-27702, doi:10.1074/jbc.M004729200 (2000).
- 79 Ma, Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* **53**, 401-426, doi:10.1146/annurev-pharmtox-011112-140320 (2013).
- 80 Al-Huseini, L. M. *et al.* Nuclear factor-erythroid 2 (NF-E2) p45-related factor-2 (Nrf2) modulates dendritic cell immune function through regulation of p38 MAPK-cAMP-responsive element binding protein/activating transcription factor 1 signaling. *J Biol Chem* **288**, 22281-22288, doi:10.1074/jbc.M113.483420 (2013).
- 81 Johnson, D. A., Amirahmadi, S., Ward, C., Fabry, Z. & Johnson, J. A. The absence of the pro-antioxidant transcription factor Nrf2 exacerbates experimental autoimmune encephalomyelitis. *Toxicol Sci* **114**, 237-246, doi:10.1093/toxsci/kfp274 (2010).
- 82 Pareek, T. K. *et al.* Triterpenoid modulation of IL-17 and Nrf-2 expression ameliorates neuroinflammation and promotes remyelination in autoimmune encephalomyelitis. *Scientific reports* **1**, 201, doi:10.1038/srep00201 (2011).
- 83 Li, B. *et al.* Sulforaphane ameliorates the development of experimental autoimmune encephalomyelitis by antagonizing oxidative stress and Th17-related inflammation in mice. *Exp Neurol* **250**, 239-249, doi:10.1016/j.expneurol.2013.10.002 (2013).
- 84 Croze, E., Yamaguchi, K. D., Knappertz, V., Reder, A. T. & Salamon, H. Interferon-beta-1b-induced short- and long-term signatures of treatment activity in multiple sclerosis. *Pharmacogenomics J* **13**, 443-451, doi:10.1038/tpj.2012.27 (2013).
- 85 Bruck, J., Dringen, R., Amasuno, A., Pau-Charles, I. & Ghoreschi, K. A review of the mechanisms of action of dimethylfumarate in the treatment of psoriasis. *Exp Dermatol* **27**, 611-624, doi:10.1111/exd.13548 (2018).
- 86 Ma, Q., Battelli, L. & Hubbs, A. F. Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. *Am J Pathol* **168**, 1960-1974, doi:10.2353/ajpath.2006.051113 (2006).
- 87 Zhao, M. *et al.* Nuclear Factor Erythroid 2-related Factor 2 Deficiency Exacerbates Lupus Nephritis in B6/lpr mice by Regulating Th17 Cell Function. *Sci Rep* **6**, 38619, doi:10.1038/srep38619 (2016).
- 88 Wu, W. J. *et al.* S-propargyl-cysteine attenuates inflammatory response in rheumatoid arthritis by modulating the Nrf2-ARE signaling pathway. *Redox Biol* **10**, 157-167, doi:10.1016/j.redox.2016.08.011 (2016).
- 89 van Wietmarschen, H. *et al.* Systems biology guided by Chinese medicine reveals new markers for sub-typing rheumatoid arthritis patients. *J Clin Rheumatol* **15**, 330-337, doi:10.1097/RHU.0b013e3181ba3926 (2009).
- 90 Guan, C. P. *et al.* The susceptibility to vitiligo is associated with NF-E2-related factor2 (Nrf2) gene polymorphisms: a study on Chinese Han population. *Exp Dermatol* **17**, 1059-1062, doi:10.1111/j.1600-0625.2008.00752.x (2008).
- 91 Song, P. *et al.* Genetic polymorphism of the Nrf2 promoter region is associated with vitiligo risk in Han Chinese populations. *J Cell Mol Med* **20**, 1840-1850, doi:10.1111/jcmm.12874 (2016).
- 92 Natarajan, V. T. *et al.* Transcriptional upregulation of Nrf2-dependent phase II detoxification genes in the involved epidermis of vitiligo vulgaris. *J Invest Dermatol* **130**, 2781-2789, doi:10.1038/jid.2010.201 (2010).

- 93 van Eeden, S. F. & Sin, D. D. Oxidative stress in chronic obstructive pulmonary disease: a lung and systemic process. *Can Respir J* **20**, 27-29, doi:10.1155/2013/509130 (2013).
- 94 Rangasamy, T. *et al.* Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest* **114**, 1248-1259, doi:10.1172/JCI21146 (2004).
- 95 Rangasamy, T. *et al.* Cigarette smoke-induced emphysema in A/J mice is associated with pulmonary oxidative stress, apoptosis of lung cells, and global alterations in gene expression. *Am J Physiol Lung Cell Mol Physiol* **296**, L888-900, doi:10.1152/ajplung.90369.2008 (2009).
- 96 Sussan, T. E. *et al.* Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. *Proc Natl Acad Sci U S A* **106**, 250-255, doi:10.1073/pnas.0804333106 (2009).
- 97 Blake, D. J. *et al.* Deletion of Keap1 in the lung attenuates acute cigarette smoke-induced oxidative stress and inflammation. *Am J Respir Cell Mol Biol* **42**, 524-536, doi:10.1165/rcmb.2009-0054OC (2010).
- 98 Goven, D. *et al.* Altered Nrf2/Keap1-Bach1 equilibrium in pulmonary emphysema. *Thorax* **63**, 916-924, doi:10.1136/thx.2007.091181 (2008).
- 99 Hua, C. C. *et al.* Functional haplotypes in the promoter region of transcription factor Nrf2 in chronic obstructive pulmonary disease. *Dis Markers* **28**, 185-193, doi:10.3233/DMA-2010-0700 (2010).
- 100 Bewley, M. A. *et al.* Opsonic Phagocytosis in Chronic Obstructive Pulmonary Disease is Enhanced by Nrf2 Agonists. *Am J Respir Crit Care Med*, doi:10.1164/rccm.201705-0903OC (2018).
- 101 Wise, R. A. *et al.* Lack of Effect of Oral Sulforaphane Administration on Nrf2 Expression in COPD: A Randomized, Double-Blind, Placebo Controlled Trial. *PLoS One* **11**, e0163716, doi:10.1371/journal.pone.0163716 (2016).
- 102 Markart, P. *et al.* Alveolar oxidative stress is associated with elevated levels of nonenzymatic low-molecular-weight antioxidants in patients with different forms of chronic fibrosing interstitial lung diseases. *Antioxid Redox Signal* **11**, 227-240, doi:10.1089/ARS.2008.2105 (2009).
- 103 Biasi, F., Leonarduzzi, G., Oteiza, P. I. & Poli, G. Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. *Antioxid Redox Signal* **19**, 1711-1747, doi:10.1089/ars.2012.4530 (2013).
- 104 Benson, A. M., Hunkeler, M. J. & Talalay, P. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proc Natl Acad Sci U S A* **77**, 5216-5220 (1980).
- 105 McMahon, M. *et al.* The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res* **61**, 3299-3307 (2001).
- 106 Kumar, V. *et al.* Novel chalcone derivatives as potent Nrf2 activators in mice and human lung epithelial cells. *J Med Chem* **54**, 4147-4159, doi:10.1021/jm2002348 (2011).
- 107 Khor, T. O. *et al.* Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. *Cancer Res* **66**, 11580-11584, doi:10.1158/0008-5472.CAN-06-3562 (2006).
- 108 Baillie, J. K. *et al.* Analysis of the human monocyte-derived macrophage transcriptome and response to lipopolysaccharide provides new insights into genetic aetiology of inflammatory bowel disease. *PLoS Genet* **13**, e1006641, doi:10.1371/journal.pgen.1006641 (2017).

Demonstrates the significance of NRF2 in human inflammatory bowel disease.

- 109 Arisawa, T. *et al.* Nrf2 gene promoter polymorphism and gastric carcinogenesis. *Hepatogastroenterology* **55**, 750-754 (2008).
  - 110 Aleksunes, L. M. & Manautou, J. E. Emerging role of Nrf2 in protecting against hepatic and gastrointestinal disease. *Toxicol Pathol* **35**, 459-473, doi:10.1080/01926230701311344 (2007).
  - 111 Okawa, H. *et al.* Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. *Biochem Biophys Res Commun* **339**, 79-88, doi:10.1016/j.bbrc.2005.10.185 (2006).
  - 112 Lamle, J. *et al.* Nuclear factor-eythroid 2-related factor 2 prevents alcohol-induced fulminant liver injury. *Gastroenterology* **134**, 1159-1168, doi:10.1053/j.gastro.2008.01.011 (2008).
  - 113 Wakabayashi, N. *et al.* Regulation of notch1 signaling by nrf2: implications for tissue regeneration. *Sci Signal* **3**, ra52, doi:10.1126/scisignal.2000762 (2010).
  - 114 Kim, J. H. *et al.* NRF2-mediated Notch pathway activation enhances hematopoietic reconstitution following myelosuppressive radiation. *J Clin Invest* **124**, 730-741, doi:10.1172/JCI70812 (2014).
  - 115 Duarte, T. L. *et al.* Genetic disruption of NRF2 promotes the development of necroinflammation and liver fibrosis in a mouse model of HFE-hereditary hemochromatosis. *Redox Biol* **11**, 157-169, doi:10.1016/j.redox.2016.11.013 (2017).
  - 116 Wasik, U., Milkiewicz, M., Kempinska-Podhorodecka, A. & Milkiewicz, P. Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in Primary Biliary Cholangitis. *Sci Rep* **7**, 44769, doi:10.1038/srep44769 (2017).
  - 117 Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813-820, doi:10.1038/414813a (2001).
  - 118 Meakin, P. J. *et al.* Susceptibility of Nrf2-Null Mice to Steatohepatitis and Cirrhosis upon Consumption of a High-Fat Diet Is Associated with Oxidative Stress, Perturbation of the Unfolded Protein Response, and Disturbance in the Expression of Metabolic Enzymes but Not with Insulin Resistance. *Mol Cell Biol* **34**, 3305-3320, doi:10.1128/MCB.00677-14 (2014).
  - 119 Uruno, A. *et al.* Nrf2-Mediated Regulation of Skeletal Muscle Glycogen Metabolism. *Mol Cell Biol* **36**, 1655-1672, doi:10.1128/MCB.01095-15 (2016).
  - 120 Uruno, A., Yagishita, Y. & Yamamoto, M. The Keap1-Nrf2 system and diabetes mellitus. *Arch Biochem Biophys* **566**, 76-84, doi:10.1016/j.abb.2014.12.012 (2015).
  - 121 Jiang, T. *et al.* The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. *Diabetes* **59**, 850-860, doi:10.2337/db09-1342 (2010).
  - 122 Sharma, R. S. *et al.* Experimental Nonalcoholic Steatohepatitis and Liver Fibrosis Are Ameliorated by Pharmacologic Activation of Nrf2 (NF-E2 p45-Related Factor 2). *Cell Mol Gastroenterol Hepatol* **5**, 367-398, doi:10.1016/j.jcmgh.2017.11.016 (2018).
- Describes that pharmacological activation of NRF2 in obese and insulin resistant mice reversed insulin resistance, suppressed hepatic steatosis and fibrosis, and that these protective effects were due to inhibition of endoplasmic reticulum (ER), inflammatory, and oxidative stress.**
- 123 Axelsson, A. S. *et al.* Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. *Sci Transl Med* **9**, doi:10.1126/scitranslmed.aah4477 (2017).
  - 124 Zheng, H. *et al.* Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. *Diabetes* **60**, 3055-3066, doi:10.2337/db11-0807 (2011).

- 125 Jimenez-Osorio, A. S. *et al.* Nrf2 and redox status in prediabetic and diabetic patients. *Int J Mol Sci* **15**, 20290-20305, doi:10.3390/ijms151120290 (2014).
- 126 Wang, X. *et al.* Association between the NF-E2 Related Factor 2 Gene Polymorphism and Oxidative Stress, Anti-Oxidative Status, and Newly-Diagnosed Type 2 Diabetes Mellitus in a Chinese Population. *Int J Mol Sci* **16**, 16483-16496, doi:10.3390/ijms160716483 (2015).
- 127 Xu, X. *et al.* Genetic variants of nuclear factor erythroid-derived 2-like 2 associated with the complications in Han descents with type 2 diabetes mellitus of Northeast China. *J Cell Mol Med* **20**, 2078-2088, doi:10.1111/jcmm.12900 (2016).
- 128 Jimenez-Osorio, A. S. *et al.* Association of Nuclear Factor-Erythroid 2-Related Factor 2, Thioredoxin Interacting Protein, and Heme Oxygenase-1 Gene Polymorphisms with Diabetes and Obesity in Mexican Patients. *Oxid Med Cell Longev* **2016**, 7367641, doi:10.1155/2016/7367641 (2016).
- 129 Palsamy, P., Ayaki, M., Elanchezian, R. & Shinohara, T. Promoter demethylation of Keap1 gene in human diabetic cataractous lenses. *Biochem Biophys Res Commun* **423**, 542-548, doi:10.1016/j.bbrc.2012.05.164 (2012).
- 130 Chowdhry, S. *et al.* Loss of Nrf2 markedly exacerbates nonalcoholic steatohepatitis. *Free Radic Biol Med* **48**, 357-371, doi:10.1016/j.freeradbiomed.2009.11.007 (2010).
- 131 Sugimoto, H. *et al.* Deletion of nuclear factor-E2-related factor-2 leads to rapid onset and progression of nutritional steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* **298**, G283-294, doi:10.1152/ajpgi.00296.2009 (2010).
- 132 Reccia, I. *et al.* Non-alcoholic fatty liver disease: A sign of systemic disease. *Metabolism* **72**, 94-108, doi:10.1016/j.metabol.2017.04.011 (2017).
- 133 Slocum, S. L. *et al.* Keap1/Nrf2 pathway activation leads to a repressed hepatic gluconeogenic and lipogenic program in mice on a high-fat diet. *Arch Biochem Biophys* **591**, 57-65, doi:10.1016/j.abb.2015.11.040 (2016).
- 134 Takahashi, Y. *et al.* Does hepatic oxidative stress enhance activation of nuclear factor-E2-related factor in patients with nonalcoholic steatohepatitis? *Antioxid Redox Signal* **20**, 538-543, doi:10.1089/ars.2013.5470 (2014).
- 135 Harrison, D., Griendling, K. K., Landmesser, U., Hornig, B. & Drexler, H. Role of oxidative stress in atherosclerosis. *Am J Cardiol* **91**, 7A-11A (2003).
- 136 Katsumata, Y. *et al.* Endogenous prostaglandin D2 and its metabolites protect the heart against ischemia-reperfusion injury by activating Nrf2. *Hypertension* **63**, 80-87, doi:10.1161/HYPERTENSIONAHA.113.01639 (2014).
- 137 Ashrafian, H. *et al.* Fumarate is cardioprotective via activation of the Nrf2 antioxidant pathway. *Cell Metab* **15**, 361-371, doi:10.1016/j.cmet.2012.01.017 (2012).

Demonstrates the beneficial effects of targeting NRF2 for cardioprotection.

- 138 Calvert, J. W. *et al.* Genetic and pharmacologic hydrogen sulfide therapy attenuates ischemia-induced heart failure in mice. *Circulation* **122**, 11-19, doi:10.1161/CIRCULATIONAHA.109.920991 (2010).
- 139 Li, J. *et al.* Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler Thromb Vasc Biol* **29**, 1843-1850, doi:10.1161/ATVBAHA.109.189480 (2009).
- 140 Collins, A. R. *et al.* Myeloid deletion of nuclear factor erythroid 2-related factor 2 increases atherosclerosis and liver injury. *Arterioscler Thromb Vasc Biol* **32**, 2839-2846, doi:10.1161/ATVBAHA.112.300345 (2012).
- 141 Ruotsalainen, A. K. *et al.* The absence of macrophage Nrf2 promotes early atherogenesis. *Cardiovasc Res* **98**, 107-115, doi:10.1093/cvr/cvt008 (2013).

- 142 Sussan, T. E. *et al.* Disruption of Nrf2, a key inducer of antioxidant defenses, attenuates ApoE-mediated atherosclerosis in mice. *PLoS One* **3**, e3791, doi:10.1371/journal.pone.0003791 (2008).
- 143 Barajas, B. *et al.* NF-E2-related factor 2 promotes atherosclerosis by effects on plasma lipoproteins and cholesterol transport that overshadow antioxidant protection. *Arterioscler Thromb Vasc Biol* **31**, 58-66, doi:10.1161/ATVBAHA.110.210906 (2011).
- 144 Freigang, S. *et al.* Nrf2 is essential for cholesterol crystal-induced inflammasome activation and exacerbation of atherosclerosis. *Eur J Immunol* **41**, 2040-2051, doi:10.1002/eji.201041316 (2011).
- 145 Ruotsalainen, A. K. *et al.* Nrf2 deficiency impairs atherosclerotic lesion development but promotes features of plaque instability in hypercholesterolemic mice. *Cardiovasc Res*, doi:10.1093/cvr/cvy143 (2018).
- 146 Tan, S. M. *et al.* Derivative of bardoxolone methyl, dh404, in an inverse dose-dependent manner lessens diabetes-associated atherosclerosis and improves diabetic kidney disease. *Diabetes* **63**, 3091-3103, doi:10.2337/db13-1743 (2014).
- 147 Xie, L. *et al.* Hydrogen Sulfide Induces Keap1 S-sulfhydration and Suppresses Diabetes-Accelerated Atherosclerosis via Nrf2 Activation. *Diabetes* **65**, 3171-3184, doi:10.2337/db16-0020 (2016).
- 148 Quiles, J. L. *et al.* Curcuma longa extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol* **22**, 1225-1231 (2002).
- 149 Harvey, P. A. & Leinwand, L. A. The cell biology of disease: cellular mechanisms of cardiomyopathy. *J Cell Biol* **194**, 355-365, doi:10.1083/jcb.201101100 (2011).
- 150 Pedruzzi, L. M. *et al.* Systemic inflammation and oxidative stress in hemodialysis patients are associated with down-regulation of Nrf2. *J Nephrol* **28**, 495-501, doi:10.1007/s40620-014-0162-0 (2015).
- 151 Shimoyama, Y., Mitsuda, Y., Tsuruta, Y., Hamajima, N. & Niwa, T. Polymorphism of Nrf2, an antioxidative gene, is associated with blood pressure and cardiovascular mortality in hemodialysis patients. *Int J Med Sci* **11**, 726-731, doi:10.7150/ijms.8590 (2014).
- 152 Kannan, S. *et al.* Nrf2 deficiency prevents reductive stress-induced hypertrophic cardiomyopathy. *Cardiovasc Res* **100**, 63-73, doi:10.1093/cvr/cvt150 (2013).
- 153 Kanninen, K. *et al.* Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **106**, 16505-16510, doi:10.1073/pnas.0908397106 (2009).
- 154 Joshi, G., Gan, K. A., Johnson, D. A. & Johnson, J. A. Increased Alzheimer's disease-like pathology in the APP/ PS1DeltaE9 mouse model lacking Nrf2 through modulation of autophagy. *Neurobiol Aging* **36**, 664-679, doi:10.1016/j.neurobiolaging.2014.09.004 (2015).
- 155 Jo, C. *et al.* Nrf2 reduces levels of phosphorylated tau protein by inducing autophagy adaptor protein NDP52. *Nature communications* **5**, 3496, doi:10.1038/ncomms4496 (2014).
- 156 Rojo, A. I. *et al.* NRF2 deficiency replicates transcriptomic changes in Alzheimer's patients and worsens APP and TAU pathology. *Redox biology* **13**, 444-451, doi:10.1016/j.redox.2017.07.006 (2017).

The first mouse model combining amyloidopathy and tauopathy with presence or absence of NRF2; demonstrates that NRF2 deficient mice exhibit pathway alterations common to patients with Alzheimer's disease.

- 157 Rojo, A. I. *et al.* Deficiency in the transcription factor NRF2 worsens inflammatory parameters in a mouse model with combined tauopathy and amyloidopathy. *Redox Biol* **18**, 173-180, doi:10.1016/j.redox.2018.07.006 (2018).
- 158 Lastres-Becker, I. *et al.* alpha-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease. *Hum Mol Genet* **21**, 3173-3192, doi:10.1093/hmg/dds143 (2012).
- 159 Lastres-Becker, I. *et al.* Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. *Antioxid Redox Signal* **25**, 61-77, doi:10.1089/ars.2015.6549 (2016).

Provides preclinical proof-of-concept indicating that DMF can be repurposed for the treatment of synucleinopathies, such as Parkinson's disease.

- 160 Gan, L., Vargas, M. R., Johnson, D. A. & Johnson, J. A. Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the alpha-synuclein mutant (A53T) mouse model. *J Neurosci* **32**, 17775-17787, doi:10.1523/JNEUROSCI.3049-12.2012 (2012).
- 161 Lastres-Becker, I. *et al.* Fractalkine activates NRF2/NFE2L2 and heme oxygenase 1 to restrain tauopathy-induced microgliosis. *Brain : a journal of neurology* **137**, 78-91, doi:10.1093/brain/awt323 (2014).
- 162 Skibinski, G. *et al.* Nrf2 mitigates LRRK2- and alpha-synuclein-induced neurodegeneration by modulating proteostasis. *Proc Natl Acad Sci U S A* **114**, 1165-1170, doi:10.1073/pnas.1522872114 (2017).
- 163 Clark, L. N. *et al.* Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. *Neurology* **67**, 1786-1791, doi:10.1212/01.wnl.0000244345.49809.36 (2006).
- 164 Chen, P. C. *et al.* Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc Natl Acad Sci U S A* **106**, 2933-2938, doi:10.1073/pnas.0813361106 (2009).
- 165 Vargas, M. R., Johnson, D. A., Sirkis, D. W., Messing, A. & Johnson, J. A. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J Neurosci* **28**, 13574-13581, doi:10.1523/JNEUROSCI.4099-08.2008 (2008).
- 166 Innamorato, N. G., Lastres-Becker, I. & Cuadrado, A. Role of microglial redox balance in modulation of neuroinflammation. *Curr Opin Neurol* **22**, 308-314, doi:10.1097/WCO.0b013e32832a3225 (2009).
- 167 Rojo, A. I. *et al.* Redox control of microglial function: molecular mechanisms and functional significance. *Antioxid Redox Signal* **21**, 1766-1801, doi:10.1089/ars.2013.5745 (2014).
- 168 Rojo, A. I. *et al.* Nrf2 regulates microglial dynamics and neuroinflammation in experimental Parkinson's disease. *Glia* **58**, 588-598, doi:10.1002/glia.20947 (2010).
- 169 van Muiswinkel, F. L. *et al.* Expression of NAD(P)H:quinone oxidoreductase in the normal and Parkinsonian substantia nigra. *Neurobiology of aging* **25**, 1253-1262, doi:10.1016/j.neurobiolaging.2003.12.010 (2004).
- 170 Schipper, H. M., Song, W., Zukor, H., Hascalogici, J. R. & Zeligman, D. Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement. *J Neurochem* **110**, 469-485, doi:10.1111/j.1471-4159.2009.06160.x (2009).
- 171 Cuadrado, A., Moreno-Murciano, P. & Pedraza-Chaverri, J. The transcription factor Nrf2 as a new therapeutic target in Parkinson's disease. *Expert Opin Ther Targets* **13**, 319-329, doi:10.1517/13543780802716501 (2009).

- 172 Bergstrom, P. *et al.* Association of NFE2L2 and KEAP1 haplotypes with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* **15**, 130-137, doi:10.3109/21678421.2013.839708 (2014).
- 173 LoGerfo, A. *et al.* Lack of association between nuclear factor erythroid-derived 2-like 2 promoter gene polymorphisms and oxidative stress biomarkers in amyotrophic lateral sclerosis patients. *Oxid Med Cell Longev* **2014**, 432626, doi:10.1155/2014/432626 (2014).
- 174 von Otter, M. *et al.* Genetic associations of Nrf2-encoding NFE2L2 variants with Parkinson's disease - a multicenter study. *BMC Med Genet* **15**, 131, doi:10.1186/s12881-014-0131-4 (2014).
- 175 Chen, Y. C., Wu, Y. R., Wu, Y. C., Lee-Chen, G. J. & Chen, C. M. Genetic analysis of NFE2L2 promoter variation in Taiwanese Parkinson's disease. *Parkinsonism Relat Disord* **19**, 247-250, doi:10.1016/j.parkreldis.2012.10.018 (2013).
- 176 Paupe, V. *et al.* Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia. *PLoS One* **4**, e4253, doi:10.1371/journal.pone.0004253 (2009).
- 177 D'Oria, V. *et al.* Frataxin deficiency leads to reduced expression and impaired translocation of NF-E2-related factor (Nrf2) in cultured motor neurons. *Int J Mol Sci* **14**, 7853-7865, doi:10.3390/ijms14047853 (2013).
- 178 Shan, Y. *et al.* Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. *Antioxid Redox Signal* **19**, 1481-1493, doi:10.1089/ars.2012.4537 (2013).
- 179 Quinti, L. *et al.* SIRT2- and NRF2-Targeting Thiazole-Containing Compound with Therapeutic Activity in Huntington's Disease Models. *Cell chemical biology* **23**, 849-861, doi:10.1016/j.chembiol.2016.05.015 (2016).
- 180 Ellrichmann, G. *et al.* Efficacy of fumaric acid esters in the R6/2 and YAC128 models of Huntington's disease. *PLoS One* **6**, e16172, doi:10.1371/journal.pone.0016172 (2011).
- 181 Stack, C. *et al.* Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington's disease. *Free Radic Biol Med* **49**, 147-158, doi:10.1016/j.freeradbiomed.2010.03.017 (2010).
- 182 Abeti, R. *et al.* Targeting lipid peroxidation and mitochondrial imbalance in Friedreich's ataxia. *Pharmacol Res* **99**, 344-350, doi:10.1016/j.phrs.2015.05.015 (2015).
- 183 Abeti, R., Baccaro, A., Esteras, N. & Giunti, P. Novel Nrf2-Inducer Prevents Mitochondrial Defects and Oxidative Stress in Friedreich's Ataxia Models. *Front Cell Neurosci* **12**, 188, doi:10.3389/fncel.2018.00188 (2018).
- 184 Kalra, S. *et al.* Highly potent activation of Nrf2 by topical tricyclic bis(cyano enone): implications for protection against UV radiation during thiopurine therapy. *Cancer Prev Res (Phila)* **5**, 973-981, doi:10.1158/1940-6207.CAPR-12-0041 (2012).
- 185 Yang, L. *et al.* Reduced formation of depurinating estrogen-DNA adducts by sulforaphane or KEAP1 disruption in human mammary epithelial MCF-10A cells. *Carcinogenesis* **34**, 2587-2592, doi:10.1093/carcin/bgt246 (2013).
- 186 DeBerardinis, R. J. & Chandel, N. S. Fundamentals of cancer metabolism. *Sci Adv* **2**, e1600200, doi:10.1126/sciadv.1600200 (2016).
- 187 DeNicola, G. M. *et al.* NRF2 regulates serine biosynthesis in non-small cell lung cancer. *Nat Genet* **47**, 1475-1481, doi:10.1038/ng.3421 (2015).
- 188 Romero, R. *et al.* Keap1 loss promotes Kras-driven lung cancer and results in dependence on glutaminolysis. *Nature medicine* **23**, 1362-1368, doi:10.1038/nm.4407 (2017).

- 189 Sayin, V. I. *et al.* Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. *eLife* **6**, doi:10.7554/eLife.28083 (2017).

Describes the mechanism by which activation of NRF2 promotes Kras-driven lung carcinogenesis and results in dependence on glutaminolysis, which can be therapeutically exploited.

- 190 Bailey, M. H. *et al.* Comprehensive Characterization of Cancer Driver Genes and Mutations. *Cell* **173**, 371-385 e318, doi:10.1016/j.cell.2018.02.060 (2018).
- 191 Kerins, M. J. & Ooi, A. A catalogue of somatic NRF2 gain-of-function mutations in cancer. *Scientific reports* **8**, 12846, doi:10.1038/s41598-018-31281-0 (2018).
- 192 Bar-Peled, L. *et al.* Chemical Proteomics Identifies Druggable Vulnerabilities in a Genetically Defined Cancer. *Cell* **171**, 696-709 e623, doi:10.1016/j.cell.2017.08.051 (2017).
- 193 Sanchez-Vega, F. *et al.* Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell* **173**, 321-337 e310, doi:10.1016/j.cell.2018.03.035 (2018).
- 194 Taguchi, K. *et al.* Genetic analysis of cytoprotective functions supported by graded expression of Keap1. *Mol Cell Biol* **30**, 3016-3026, doi:10.1128/MCB.01591-09 (2010).
- 195 Best, S. A. *et al.* Synergy between the KEAP1/NRF2 and PI3K Pathways Drives Non-Small-Cell Lung Cancer with an Altered Immune Microenvironment. *Cell Metab* **27**, 935-943 e934, doi:10.1016/j.cmet.2018.02.006 (2018).

Demonstrates that mice with constitutive NRF2 activation do not develop spontaneous tumours, but that the combined activation of the PI3K pathway and NRF2 promotes the development of lung cancer characterized by immunosuppressive microenvironment, which can be therapeutically exploited by use of immune checkpoint inhibitors.

- 196 DeNicola, G. M. *et al.* Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* **475**, 106-109, doi:10.1038/nature10189 (2011).

Describes that NRF2 is activated by oncogenes to promote ROS detoxification.

- 197 Chartoumpakis, D. V. *et al.* Nrf2 prevents Notch-induced insulin resistance and tumorigenesis in mice. *JCI Insight* **3**, doi:10.1172/jci.insight.97735 (2018).
- 198 Knatko, E. V. *et al.* Whole-Exome Sequencing Validates a Preclinical Mouse Model for the Prevention and Treatment of Cutaneous Squamous Cell Carcinoma. *Cancer Prev Res (Phila)* **10**, 67-75, doi:10.1158/1940-6207.CAPR-16-0218 (2017).
- 199 Knatko, E. V., Higgins, M., Fahey, J. W. & Dinkova-Kostova, A. T. Loss of Nrf2 abrogates the protective effect of Keap1 downregulation in a preclinical model of cutaneous squamous cell carcinoma. *Scientific reports* **6**, 25804, doi:10.1038/srep25804 (2016).
- 200 Satoh, H. *et al.* NRF2 Intensifies Host Defense Systems to Prevent Lung Carcinogenesis, but After Tumor Initiation Accelerates Malignant Cell Growth. *Cancer Res* **76**, 3088-3096, doi:10.1158/0008-5472.CAN-15-1584 (2016).
- 201 Satoh, H., Moriguchi, T., Takai, J., Ebina, M. & Yamamoto, M. Nrf2 prevents initiation but accelerates progression through the Kras signaling pathway during lung carcinogenesis. *Cancer Res* **73**, 4158-4168, doi:10.1158/0008-5472.CAN-12-4499 (2013).
- 202 Kensler, T. W. & Wakabayashi, N. Nrf2: friend or foe for chemoprevention? *Carcinogenesis* **31**, 90-99, doi:10.1093/carcin/bgp231 (2010).
- 203 Linker, R. A. *et al.* Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* **134**, 678-692, doi:10.1093/brain/awq386 (2011).
- 204 Spencer, S. R., Wilczak, C. A. & Talalay, P. Induction of glutathione transferases and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues. *Cancer Res* **50**, 7871-7875 (1990).
- 205 Fox, R. J. *et al.* Efficacy and Tolerability of Delayed-release Dimethyl Fumarate in Black, Hispanic, and Asian Patients with Relapsing-Remitting Multiple Sclerosis: Post Hoc

Integrated Analysis of DEFINE and CONFIRM. *Neurol Ther* **6**, 175-187, doi:10.1007/s40120-017-0077-5 (2017).

206 Fernandez, O. *et al.* Efficacy and Safety of Delayed-release Dimethyl Fumarate for Relapsing-remitting Multiple Sclerosis in Prior Interferon Users: An Integrated Analysis of DEFINE and CONFIRM. *Clin Ther* **39**, 1671-1679, doi:10.1016/j.clinthera.2017.06.012 (2017).

207 Chen, H. *et al.* Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. *J Clin Invest* **124**, 2188-2192, doi:10.1172/JCI72151 (2014).

208 Zhang, Y., Talalay, P., Cho, C. G. & Posner, G. H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci U S A* **89**, 2399-2403 (1992).

The first study describing the identification of sulforaphane as a highly potent naturally occurring inducer of cytoprotective enzymes.

209 Zhang, D. D. & 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* **23**, 8137-8151 (2003).

The first study that identified C151 as a major sensor cysteine in KEAP1.

210 Holmstrom, K. M., Kostov, R. V. & Dinkova-Kostova, A. T. The multifaceted role of Nrf2 in mitochondrial function. *Curr Opin Toxicol* **1**, 80-91, doi:10.1016/j.cotox.2016.10.002 (2016).

211 Singh, K. *et al.* Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci U S A* **111**, 15550-15555, doi:10.1073/pnas.1416940111 (2014).

212 Fahey, J. W., Zhang, Y. & Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci U S A* **94**, 10367-10372 (1997).

213 Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K. & Talalay, P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. *Cancer Epidemiol Biomarkers Prev* **7**, 1091-1100 (1998).

214 Fahey, J. W. *et al.* Protection of humans by plant glucosinolates: efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prev Res (Phila)* **5**, 603-611, doi:10.1158/1940-6207.CAPR-11-0538 (2012).

215 Cipolla, B. G. *et al.* Effect of Sulforaphane in Men with Biochemical Recurrence after Radical Prostatectomy. *Cancer Prev Res (Phila)* **8**, 712-719, doi:10.1158/1940-6207.CAPR-14-0459 (2015).

216 Fahey, J. W. *et al.* Stabilized sulforaphane for clinical use: Phytochemical delivery efficiency. *Mol Nutr Food Res* **61**, doi:10.1002/mnfr.201600766 (2017).

217 Bent, S. *et al.* Identification of urinary metabolites that correlate with clinical improvements in children with autism treated with sulforaphane from broccoli. *Mol Autism* **9**, 35, doi:10.1186/s13229-018-0218-4 (2018).

218 Sporn, M. B. *et al.* New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress. *J Nat Prod* **74**, 537-545, doi:10.1021/np100826q (2011).

219 Honda, T. *et al.* Synthetic oleanane and ursane triterpenoids with modified rings A and C: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *J Med Chem* **43**, 4233-4246 (2000).

220 Dinkova-Kostova, A. T. *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* **102**, 4584-4589, doi:10.1073/pnas.0500815102 (2005).

One of the earliest studies that identified the cyanoenone triterpenoids as extremely potent NRF2 activators.

- 221 Shekh-Ahmad, T. *et al.* KEAP1 inhibition is neuroprotective and suppresses the development of epilepsy. *Brain* **141**, 1390-1403, doi:10.1093/brain/awy071 (2018).
- 222 Ruiz, S., Pergola, P. E., Zager, R. A. & Vaziri, N. D. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int* **83**, 1029-1041, doi:10.1038/ki.2012.439 (2013).
- 223 Ding, Y. *et al.* The synthetic triterpenoid, RTA 405, increases the glomerular filtration rate and reduces angiotensin II-induced contraction of glomerular mesangial cells. *Kidney Int* **83**, 845-854, doi:10.1038/ki.2012.393 (2013).
- 224 Yamaguchi, J., Tanaka, T. & Nangaku, M. Recent advances in understanding of chronic kidney disease. *F1000Res* **4**, doi:10.12688/f1000research.6970.1 (2015).
- 225 Sutendra, G. & Michelakis, E. D. The metabolic basis of pulmonary arterial hypertension. *Cell Metab* **19**, 558-573, doi:10.1016/j.cmet.2014.01.004 (2014).
- 226 Chung, L. *et al.* Survival and predictors of mortality in systemic sclerosis-associated pulmonary arterial hypertension: outcomes from the pulmonary hypertension assessment and recognition of outcomes in scleroderma registry. *Arthritis Care Res (Hoboken)* **66**, 489-495, doi:10.1002/acr.22121 (2014).
- 227 Kearney, M., Orrell, R. W., Fahey, M. & Pandolfo, M. Antioxidants and other pharmacological treatments for Friedreich ataxia. *Cochrane Database Syst Rev*, CD007791, doi:10.1002/14651858.CD007791.pub3 (2012).
- 228 Freeman, B. A., O'Donnell, V. B. & Schopfer, F. J. The discovery of nitro-fatty acids as products of metabolic and inflammatory reactions and mediators of adaptive cell signaling. *Nitric Oxide* **77**, 106-111, doi:10.1016/j.niox.2018.05.002 (2018).
- 229 Baker, L. M. *et al.* Nitro-fatty acid reaction with glutathione and cysteine. Kinetic analysis of thiol alkylation by a Michael addition reaction. *J Biol Chem* **282**, 31085-31093, doi:10.1074/jbc.M704085200 (2007).
- 230 Batthyany, C. *et al.* Reversible post-translational modification of proteins by nitrated fatty acids in vivo. *J Biol Chem* **281**, 20450-20463, doi:10.1074/jbc.M602814200 (2006).
- 231 Kansanen, E. *et al.* Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. *J Biol Chem* **286**, 14019-14027, doi:10.1074/jbc.M110.190710 (2011).
- 232 Deen, A. J. *et al.* Regulation of stress signaling pathways by nitro-fatty acids. *Nitric Oxide*, doi:10.1016/j.niox.2018.03.012 (2018).
- 233 Rudolph, V. *et al.* Nitro-fatty acid metabolome: saturation, desaturation, beta-oxidation, and protein adduction. *J Biol Chem* **284**, 1461-1473, doi:10.1074/jbc.M802298200 (2009).
- 234 Schopfer, F. J., Vitturi, D. A., Jorkasky, D. K. & Freeman, B. A. Nitro-fatty acids: New drug candidates for chronic inflammatory and fibrotic diseases. *Nitric Oxide*, doi:10.1016/j.niox.2018.06.006 (2018).
- 235 Rodriguez-Duarte, J. *et al.* Electrophilic nitroalkene-tocopherol derivatives: synthesis, physicochemical characterization and evaluation of anti-inflammatory signaling responses. *Scientific reports* **8**, 12784, doi:10.1038/s41598-018-31218-7 (2018).
- 236 Hirotsu, Y., Katsuoka, F., Itoh, K. & Yamamoto, M. Nrf2 degron-fused reporter system: a new tool for specific evaluation of Nrf2 inducers. *Genes Cells* **16**, 406-415, doi:10.1111/j.1365-2443.2011.01496.x (2011).
- 237 Higashi, C. *et al.* The novel Nrf2 inducer TFM-735 ameliorates experimental autoimmune encephalomyelitis in mice. *Eur J Pharmacol* **802**, 76-84, doi:10.1016/j.ejphar.2017.02.044 (2017).

- 238 Schmoll, D., Engel, C. K. & Glombik, H. The Keap1-Nrf2 protein-protein interaction: A suitable target for small molecules. *Drug Discov Today Technol* **24**, 11-17, doi:10.1016/j.ddtec.2017.10.001 (2017).
- 239 Pallesen, J. S., Tran, K. T. & Bach, A. Non-covalent Small-Molecule Kelch-like ECH-Associated Protein 1-Nuclear Factor Erythroid 2-Related Factor 2 (Keap1-Nrf2) Inhibitors and Their Potential for Targeting Central Nervous System Diseases. *J Med Chem*, doi:10.1021/acs.jmedchem.8b00358 (2018).
- 240 Georgakopoulos, N. D. *et al.* Reversible Keap1 inhibitors are preferential pharmacological tools to modulate cellular mitophagy. *Scientific reports* **7**, 10303, doi:10.1038/s41598-017-07679-7 (2017).
- 241 Beamer, L. J., Li, X., Bottoms, C. A. & Hannink, M. Conserved solvent and side-chain interactions in the 1.35 Angstrom structure of the Kelch domain of Keap1. *Acta Crystallogr D Biol Crystallogr* **61**, 1335-1342, doi:10.1107/S0907444905022626 (2005).
- 242 Padmanabhan, B. *et al.* Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. *Mol Cell* **21**, 689-700, doi:10.1016/j.molcel.2006.01.013 (2006).
- 243 Tong, K. I. *et al.* Different electrostatic potentials define ETGE and DLG motifs as hinge and latch in oxidative stress response. *Mol Cell Biol* **27**, 7511-7521, doi:10.1128/MCB.00753-07 (2007).
- 244 Marcotte, D. *et al.* Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism. *Bioorg Med Chem* **21**, 4011-4019, doi:10.1016/j.bmc.2013.04.019 (2013).

One of the earliest studies describing a small molecule that inhibits the KEAP1-NRF2 protein-protein interaction.

- 245 Jiang, Z. Y. *et al.* Discovery of potent Keap1-Nrf2 protein-protein interaction inhibitor based on molecular binding determinants analysis. *J Med Chem* **57**, 2736-2745, doi:10.1021/jm5000529 (2014).
- 246 Davies, T. G. *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* **59**, 3991-4006, doi:10.1021/acs.jmedchem.6b00228 (2016).
- 247 Hu, L. *et al.* Discovery of a small-molecule inhibitor and cellular probe of Keap1-Nrf2 protein-protein interaction. *Bioorg Med Chem Lett* **23**, 3039-3043, doi:10.1016/j.bmcl.2013.03.013 (2013).

One of the earliest studies describing a small molecule that inhibits the KEAP1-NRF2 protein-protein interaction.

- 248 Jnoff, E. *et al.* Binding mode and structure-activity relationships around direct inhibitors of the Nrf2-Keap1 complex. *ChemMedChem* **9**, 699-705, doi:10.1002/cmdc.201300525 (2014).
- 249 Satoh, M. *et al.* Multiple binding modes of a small molecule to human Keap1 revealed by X-ray crystallography and molecular dynamics simulation. *FEBS Open Bio* **5**, 557-570, doi:10.1016/j.fob.2015.06.011 (2015).
- 250 Bertrand, H. 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* **58**, 7186-7194, doi:10.1021/acs.jmedchem.5b00602 (2015).
- 251 Jiang, Z. Y. *et al.* Structure-Activity and Structure-Property Relationship and Exploratory in Vivo Evaluation of the Nanomolar Keap1-Nrf2 Protein-Protein Interaction Inhibitor. *J Med Chem* **58**, 6410-6421, doi:10.1021/acs.jmedchem.5b00185 (2015).

- 252 Lu, M. C. *et al.* An inhibitor of the Keap1-Nrf2 protein-protein interaction protects NCM460 colonic cells and alleviates experimental colitis. *Sci Rep* **6**, 26585, doi:10.1038/srep26585 (2016).
- 253 Shimozone, R. *et al.* Nrf2 activators attenuate the progression of nonalcoholic steatohepatitis-related fibrosis in a dietary rat model. *Mol Pharmacol* **84**, 62-70, doi:10.1124/mol.112.084269 (2013).
- 254 Yu, S. *et al.* Nrf2 expression is regulated by epigenetic mechanisms in prostate cancer of TRAMP mice. *PLoS One* **5**, e8579, doi:10.1371/journal.pone.0008579 (2010).
- 255 Alam, M. M. *et al.* Glucocorticoid receptor signaling represses the antioxidant response by inhibiting histone acetylation mediated by the transcriptional activator NRF2. *J Biol Chem* **292**, 7519-7530, doi:10.1074/jbc.M116.773960 (2017).
- 256 Malloy, M. T. *et al.* Trafficking of the transcription factor Nrf2 to promyelocytic leukemia-nuclear bodies: implications for degradation of NRF2 in the nucleus. *J Biol Chem* **288**, 14569-14583, doi:10.1074/jbc.M112.437392 (2013).
- 257 Sun, Z., Chin, Y. E. & Zhang, D. D. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. *Mol Cell Biol* **29**, 2658-2672, doi:10.1128/MCB.01639-08 (2009).
- 258 Rada, P. *et al.* SCF/ β -TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. *Mol Cell Biol* **31**, 1121-1133, doi:10.1128/MCB.01204-10 (2011).

One of the earliest studies that describes the regulation of NRF2 by GSK3/ β -TrCP-mediated degradation.

- 259 Rada, P. *et al.* Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/ β -TrCP axis. *Mol Cell Biol* **32**, 3486-3499, doi:10.1128/MCB.00180-12 (2012).
- 260 Chowdhry, S. *et al.* Nrf2 is controlled by two distinct β -TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. *Oncogene* **32**, 3765-3781, doi:10.1038/onc.2012.388 (2013).
- 261 Wu, T. *et al.* Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. *Genes Dev* **28**, 708-722, doi:10.1101/gad.238246.114 (2014).
- 262 Tebay, L. E. *et al.* Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic Biol Med* **88**, 108-146, doi:10.1016/j.freeradbiomed.2015.06.021 (2015).
- 263 Katsuoka, F. & Yamamoto, M. Small Maf proteins (MafF, MafG, MafK): History, structure and function. *Gene* **586**, 197-205, doi:10.1016/j.gene.2016.03.058 (2016).
- 264 Attucks, O. C. *et al.* Induction of heme oxygenase I (HMOX1) by HPP-4382: a novel modulator of Bach1 activity. *PLoS One* **9**, e101044, doi:10.1371/journal.pone.0101044 (2014).
- 265 Riedl, M. A., Saxon, A. & Diaz-Sanchez, D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. *Clin Immunol* **130**, 244-251, doi:10.1016/j.clim.2008.10.007 (2009).
- 266 Bauman, J. E. *et al.* Prevention of Carcinogen-Induced Oral Cancer by Sulforaphane. *Cancer Prev Res (Phila)* **9**, 547-557, doi:10.1158/1940-6207.CAPR-15-0290 (2016).
- 267 Hammer, A. *et al.* The NRF2 pathway as potential biomarker for dimethyl fumarate treatment in multiple sclerosis. *Ann Clin Transl Neurol* **5**, 668-676, doi:10.1002/acn3.553 (2018).

- 268 Egner, P. A. *et al.* Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China. *Cancer Prev Res (Phila)* **7**, 813-823, doi:10.1158/1940-6207.CAPR-14-0103 (2014).

Demonstrated that broccoli sprout beverages can be used as frugal medicine to accelerate the detoxification of airborne pollutants in humans.

- 269 Kensler, T. W. *et al.* Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis* **33**, 101-107, doi:10.1093/carcin/bgr229 (2012).
- 270 Kostov, R. V. *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* **465**, 402-407, doi:10.1016/j.bbrc.2015.08.016 (2015).
- 271 Jusko, W. J. Moving from basic toward systems pharmacodynamic models. *J Pharm Sci* **102**, 2930-2940, doi:10.1002/jps.23590 (2013).
- 272 Dayneka, N. L., Garg, V. & Jusko, W. J. Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm* **21**, 457-478 (1993).
- 273 Egger, A. L., Small, E., Hannink, M. & Mesecar, A. D. Cul3-mediated Nrf2 ubiquitination and antioxidant response element (ARE) activation are dependent on the partial molar volume at position 151 of Keap1. *Biochem J* **422**, 171-180, doi:10.1042/BJ20090471 (2009).
- 274 Cleasby, A. *et al.* Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. *PLoS One* **9**, e98896, doi:10.1371/journal.pone.0098896 (2014).

Reports the crystal structure of the BTB domain of KEAP1 in complex with a cyanoenone triterpenoid.

- 275 Rachakonda, G. *et al.* Covalent modification at Cys151 dissociates the electrophile sensor Keap1 from the ubiquitin ligase CUL3. *Chem Res Toxicol* **21**, 705-710, doi:10.1021/tx700302s (2008).
- 276 Couch, R. D. *et al.* Studies on the reactivity of CDDO, a promising new chemopreventive and chemotherapeutic agent: implications for a molecular mechanism of action. *Bioorg Med Chem Lett* **15**, 2215-2219, doi:10.1016/j.bmcl.2005.03.031 (2005).
- 277 Zheng, S. *et al.* Synthesis, chemical reactivity as Michael acceptors, and biological potency of monocyclic cyanoenones, novel and highly potent anti-inflammatory and cytoprotective agents. *J Med Chem* **55**, 4837-4846, doi:10.1021/jm3003922 (2012).
- 278 Bradshaw, J. M. *et al.* Prolonged and tunable residence time using reversible covalent kinase inhibitors. *Nat Chem Biol* **11**, 525-531, doi:10.1038/nchembio.1817 (2015).
- 279 Kobayashi, M. *et al.* The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol Cell Biol* **29**, 493-502, doi:10.1128/MCB.01080-08 (2009).
- 280 Dayalan Naidu, S. *et al.* C151 in KEAP1 is the main cysteine sensor for the cyanoenone class of NRF2 activators, irrespective of molecular size or shape. *Scientific reports* **8**, 8037, doi:10.1038/s41598-018-26269-9 (2018).
- 281 Hayes, J. D. & Dinkova-Kostova, A. T. Oncogene-Stimulated Congestion at the KEAP1 Stress Signaling Hub Allows Bypass of NRF2 and Induction of NRF2-Target Genes that Promote Tumor Survival. *Cancer Cell* **32**, 539-541, doi:10.1016/j.ccell.2017.10.009 (2017).
- 282 van der Worp, H. B. *et al.* Can animal models of disease reliably inform human studies? *PLoS Med* **7**, e1000245, doi:10.1371/journal.pmed.1000245 (2010).

- 283 Suh, J. H. *et al.* Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci U S A* **101**, 3381-3386, doi:10.1073/pnas.0400282101 (2004).
- 284 Corenblum, M. J. *et al.* Reduced Nrf2 expression mediates the decline in neural stem cell function during a critical middle-age period. *Aging Cell* **15**, 725-736, doi:10.1111/ace.12482 (2016).
- 285 Tarantini, S. *et al.* Nrf2 Deficiency Exacerbates Obesity-Induced Oxidative Stress, Neurovascular Dysfunction, Blood-Brain Barrier Disruption, Neuroinflammation, Amyloidogenic Gene Expression, and Cognitive Decline in Mice, Mimicking the Aging Phenotype. *J Gerontol A Biol Sci Med Sci* **73**, 853-863, doi:10.1093/gerona/glx177 (2018).
- 286 Yates, M. S. *et al.* Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice. *Carcinogenesis* **30**, 1024-1031, doi:10.1093/carcin/bgp100 (2009).

One of the earliest studies showing that NRF2 regulates genes involved in lipid metabolism.

- 287 Probst, B. L., McCauley, L., Trevino, I., Wigley, W. C. & Ferguson, D. A. Cancer Cell Growth Is Differentially Affected by Constitutive Activation of NRF2 by KEAP1 Deletion and Pharmacological Activation of NRF2 by the Synthetic Triterpenoid, RTA 405. *PLoS One* **10**, e0135257, doi:10.1371/journal.pone.0135257 (2015).
- 288 Pakpoor, J. *et al.* No evidence for higher risk of cancer in patients with multiple sclerosis taking cladribine. *Neurol Neuroimmunol Neuroinflamm* **2**, e158, doi:10.1212/NXI.0000000000000158 (2015).
- 289 Kornberg, M. D. *et al.* Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science* **360**, 449-453, doi:10.1126/science.aan4665 (2018).
- 290 Yun, J. *et al.* Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* **350**, 1391-1396, doi:10.1126/science.aaa5004 (2015).
- 291 Ki, S. H., Cho, I. J., Choi, D. W. & Kim, S. G. Glucocorticoid receptor (GR)-associated SMRT binding to C/EBPbeta TAD and Nrf2 Neh4/5: role of SMRT recruited to GR in GSTA2 gene repression. *Mol Cell Biol* **25**, 4150-4165, doi:10.1128/MCB.25.10.4150-4165.2005 (2005).
- 292 Wang, X. J., Hayes, J. D., Henderson, C. J. & Wolf, C. R. Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha. *Proc Natl Acad Sci U S A* **104**, 19589-19594, doi:10.1073/pnas.0709483104 (2007).

One of the first studies to identify an NRF2 inhibitor.

- 293 Wang, H. *et al.* RXRalpha inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2. *Cancer Res* **73**, 3097-3108, doi:10.1158/0008-5472.CAN-12-3386 (2013).
- 294 Ren, D. *et al.* Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. *Proc Natl Acad Sci U S A* **108**, 1433-1438, doi:10.1073/pnas.1014275108 (2011).
- 295 Vartanian, S. *et al.* Application of Mass Spectrometry Profiling to Establish Brusatol as an Inhibitor of Global Protein Synthesis. *Mol Cell Proteomics* **15**, 1220-1231, doi:10.1074/mcp.M115.055509 (2016).
- 296 Harder, B. *et al.* Brusatol overcomes chemoresistance through inhibition of protein translation. *Mol Carcinog* **56**, 1493-1500, doi:10.1002/mc.22609 (2017).
- 297 Tsuchida, K. *et al.* Halofuginone enhances the chemo-sensitivity of cancer cells by suppressing NRF2 accumulation. *Free Radic Biol Med* **103**, 236-247, doi:10.1016/j.freeradbiomed.2016.12.041 (2017).

- 298 Tang, X. *et al.* Luteolin inhibits Nrf2 leading to negative regulation of the Nrf2/ARE pathway and sensitization of human lung carcinoma A549 cells to therapeutic drugs. *Free Radic Biol Med* **50**, 1599-1609, doi:10.1016/j.freeradbiomed.2011.03.008 (2011).
- 299 Zuo, Q. *et al.* The dietary flavone luteolin epigenetically activates the Nrf2 pathway and blocks cell transformation in human colorectal cancer HCT116 cells. *J Cell Biochem*, doi:10.1002/jcb.27275 (2018).
- 300 Ashaari, Z. *et al.* The Flavone Luteolin Improves Central Nervous System Disorders by Different Mechanisms: A Review. *J Mol Neurosci* **65**, 491-506, doi:10.1007/s12031-018-1094-2 (2018).
- 301 Zhong, Y. *et al.* Drug resistance associates with activation of Nrf2 in MCF-7/DOX cells, and wogonin reverses it by down-regulating Nrf2-mediated cellular defense response. *Mol Carcinog* **52**, 824-834, doi:10.1002/mc.21921 (2013).
- 302 Khan, N. M. *et al.* Wogonin, a plant derived small molecule, exerts potent anti-inflammatory and chondroprotective effects through the activation of ROS/ERK/Nrf2 signaling pathways in human Osteoarthritis chondrocytes. *Free radical biology & medicine* **106**, 288-301, doi:10.1016/j.freeradbiomed.2017.02.041 (2017).
- 303 Boettler, U. *et al.* Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. *J Nutr Biochem* **22**, 426-440, doi:10.1016/j.jnutbio.2010.03.011 (2011).
- 304 Arlt, A. *et al.* Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity. *Oncogene* **32**, 4825-4835, doi:10.1038/onc.2012.493 (2013).
- 305 Singh, A. *et al.* Small Molecule Inhibitor of NRF2 Selectively Intervenes Therapeutic Resistance in KEAP1-Deficient NSCLC Tumors. *ACS Chem Biol* **11**, 3214-3225, doi:10.1021/acschembio.6b00651 (2016).
- 306 Manna, A. *et al.* The variable chemotherapeutic response of Malabaricone-A in leukemic and solid tumor cell lines depends on the degree of redox imbalance. *Phytomedicine* **22**, 713-723, doi:10.1016/j.phymed.2015.05.007 (2015).
- 307 Marin-Kuan, M. *et al.* A toxicogenomics approach to identify new plausible epigenetic mechanisms of ochratoxin a carcinogenicity in rat. *Toxicol Sci* **89**, 120-134, doi:10.1093/toxsci/kfj017 (2006).
- 308 Bollong, M. J. *et al.* A Small Molecule Inhibits Deregulated NRF2 Transcriptional Activity in Cancer. *ACS Chem Biol* **10**, 2193-2198, doi:10.1021/acschembio.5b00448 (2015).
- 309 Mrowietz, U., Christophers, E. & Altmeyer, P. Treatment of psoriasis with fumaric acid esters: results of a prospective multicentre study. German Multicentre Study. *Br J Dermatol* **138**, 456-460 (1998).
- 310 Nakagami, Y. *et al.* Novel Nrf2 activators from microbial transformation products inhibit blood-retinal barrier permeability in rabbits. *Br J Pharmacol* **172**, 1237-1249, doi:10.1111/bph.12999 (2015).
- 311 Swanson, H. I. & Perdew, G. H. Half-life of aryl hydrocarbon receptor in Hepa 1 cells: evidence for ligand-dependent alterations in cytosolic receptor levels. *Arch Biochem Biophys* **302**, 167-174 (1993).
- 312 Sandoval, P. C. *et al.* Proteome-wide measurement of protein half-lives and translation rates in vasopressin-sensitive collecting duct cells. *J Am Soc Nephrol* **24**, 1793-1805, doi:10.1681/ASN.2013030279 (2013).

- 313 Emi, Y., Omura, S., Ikushiro, S. & Iyanagi, T. Accelerated degradation of mislocalized UDP-glucuronosyltransferase family 1 (UGT1) proteins in Gunn rat hepatocytes. *Arch Biochem Biophys* **405**, 163-169 (2002).
- 314 Fukuda, Y. *et al.* Conserved intramolecular disulfide bond is critical to trafficking and fate of ATP-binding cassette (ABC) transporters ABCB6 and sulfonylurea receptor 1 (SUR1)/ABCC8. *J Biol Chem* **286**, 8481-8492, doi:10.1074/jbc.M110.174516 (2011).
- 315 Schwanhaussner, B. *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337-342, doi:10.1038/nature10098 (2011).
- 316 Kato, H., Sakaki, K. & Mihara, K. Ubiquitin-proteasome-dependent degradation of mammalian ER stearyl-CoA desaturase. *J Cell Sci* **119**, 2342-2353, doi:10.1242/jcs.02951 (2006).
- 317 Srivastava, K. K., Cable, E. E., Donohue, S. E. & Bonkovsky, H. L. Molecular basis for heme-dependent induction of heme oxygenase in primary cultures of chick embryo hepatocytes. Demonstration of acquired refractoriness to heme. *Eur J Biochem* **213**, 909-917 (1993).
- 318 Ding, B., Gibbs, P. E., Brookes, P. S. & Maines, M. D. The coordinated increased expression of biliverdin reductase and heme oxygenase-2 promotes cardiomyocyte survival: a reductase-based peptide counters beta-adrenergic receptor ligand-mediated cardiac dysfunction. *FASEB J* **25**, 301-313, doi:10.1096/fj.10-166454 (2011).
- 319 Crooks, D. R., Ghosh, M. C., Haller, R. G., Tong, W. H. & Rouault, T. A. Posttranslational stability of the heme biosynthetic enzyme ferrochelatase is dependent on iron availability and intact iron-sulfur cluster assembly machinery. *Blood* **115**, 860-869, doi:10.1182/blood-2009-09-243105 (2010).
- 320 Katoh, Y. *et al.* Evolutionary conserved N-terminal domain of Nrf2 is essential for the Keap1-mediated degradation of the protein by proteasome. *Arch Biochem Biophys* **433**, 342-350, doi:10.1016/j.abb.2004.10.012 (2005).
- 321 McMahon, M., Thomas, N., Itoh, K., Yamamoto, M. & Hayes, J. D. Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. *J Biol Chem* **279**, 31556-31567, doi:10.1074/jbc.M403061200 (2004).
- 322 Massey, A. C., Follenzi, A., Kiffin, R., Zhang, C. & Cuervo, A. M. Early cellular changes after blockage of chaperone-mediated autophagy. *Autophagy* **4**, 442-456 (2008).
- 323 Bjorkoy, G. *et al.* p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol* **171**, 603-614, doi:10.1083/jcb.200507002 (2005).
- 324 Ferrington, D. A. & Gregerson, D. S. Immunoproteasomes: structure, function, and antigen presentation. *Prog Mol Biol Transl Sci* **109**, 75-112, doi:10.1016/B978-0-12-397863-9.00003-1 (2012).
- 325 Zhang, Y. *et al.* Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. *Neuron* **89**, 37-53, doi:10.1016/j.neuron.2015.11.013 (2016).
- 326 Zhang, Y. *et al.* An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* **34**, 11929-11947, doi:10.1523/JNEUROSCI.1860-14.2014 (2014).
- 327 Bagger, F. O. *et al.* BloodSpot: a database of gene expression profiles and transcriptional programs for healthy and malignant haematopoiesis. *Nucleic Acids Res* **44**, D917-924, doi:10.1093/nar/gkv1101 (2016).

Table 1. Summary of the NRF2 drug discovery pipeline sponsored by biopharmaceutical companies. Abbreviations: ALS, amyotrophic lateral sclerosis; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CTD, connective tissue disease; ER⁺, estrogen receptor positive; FRDA, Friedreich's ataxia; FTD, frontotemporal dementia; HD, Huntington's disease; IgA, immunoglobulin A; ILD, interstitial lung disease; ND, neurodegenerative diseases; PAH, pulmonary arterial hypertension; PD, Parkinson's disease; T2DM, type 2 diabetes mellitus.

Company/ Molecule	Disease	Development stage	Comments	Trial /Reference
BIOGEN: Dimethyl fumarate (DMF)	Multiple Sclerosis	Marketed	First marketed NRF2-targeting drug. Commercial name Tecfidera. Approved by FDA in 2013.	PROTEC/ NCT01930708
	Psoriasis	Marketed	Approved in Germany in 1994. Commercial name Fumaderm.	Ref. ³⁰⁹
BIOGEN: ALK8700/BII089	Multiple Sclerosis	Phase 3	Prodrug of monomethyl fumarate. Evaluation of tolerability of ALKS8700 and DMF. Estimated completion in 2019.	EVOLVE-MS-2/ NCT03093324
REATA PHARMACEUTICALS: Bardoxolone methyl (BARD, RTA402)	CTD-PAH	Phase 3	Leading synthetic triterpenoid. Efficacy and safety in CTD-PAH. Estimated completion in 2020.	CATALYST/ NCT02657356
	Pulmonary hypertension-ILD	Phase 3	Leading synthetic triterpenoid. Long-term safety and tolerability in pulmonary hypertension. Estimated completion in 2021.	RANGER/ NCT03068130
	Alport syndrome	Phase 2/3	Leading synthetic triterpenoid. Safety and efficacy. Estimated completion in 2019.	CARDINAL/ NCT03019185
	Autosomal Dominant Polycystic Kidney Disease, IgA Nephropathy, Type 1 Diabetes, Focal Segmental Glomerulosclerosis	Phase 2	Leading synthetic triterpenoid. Safety and efficacy in patients with rare chronic kidney diseases. Estimated completion in 2019.	PHOENIX/ NCT03366337
REATA PHARMACEUTICALS: Omaveloxolone (RTA408)	FRDA	Phase 2	Second-generation synthetic triterpenoid. Safety, efficacy, and pharmacodynamics. Estimated completion in 2020.	MOXIe/ NCT02255435
KYOWA HAKKO KIRIN: Bardoxolone methyl (BARD, RTA402)	T2DM, CKD	Phase 2 (completed)	Leading synthetic triterpenoid. Demonstrated increases in directly-measured glomerular filtration rate. Received the Japanese SAKIGAKE Designation for the treatment of diabetic kidney disease.	TSUBAKI/ NCT02316821
		Phase 3	Leading synthetic triterpenoid. Primary end point: time to onset of a $\geq 30\%$ decrease in estimated glomerular filtration rate from baseline or end-stage renal disease. Estimated completion in 2022.	AYAME/ NCT03550443
EVGEN PHARMA: SFX01	Subarachnoid Hemorrhage	Phase 2	Cyclodextrin-encapsulated sulforaphane. Safety, tolerability, pharmacokinetics and pharmacodynamics. Estimated completion in 2019.	SAS/ NCT02614742
	ER ⁺ Metastatic Breast Cancer	Phase 2	Cyclodextrin-encapsulated sulforaphane. Safety and efficacy when used in combination with aromatase inhibitors, tamoxifen and fulvestrant. Estimated completion in 2019.	STEM/ NCT02970682
COMPLEXA: CXA10	Primary Focal Segmental Glomerulosclerosis	Phase 2	10-nitro-octadec-9-enoic acid, a regio-isomer of nitro-oleic acid. Two titration regimens of oral CXA-10. Estimated completion in 2019.	FIRSTx/ NCT03422510

	Pulmonary Arterial Hypertension	Phase 2	10-nitro-octadec-9-enoic acid, a regio-isomer of nitro-oleic acid. Safety, efficacy and pharmacokinetics of CXA-10 on stable background therapy. Estimated completion in 2019.	PRIMEx/ NCT03449524
ARBOR PHARMACEUTICALS: XP23829	Psoriasis	Phase 2 (completed)	Tepilamide fumarate. Positive results disclosed in 2015 on efficacy as a potential treatment for moderate-to-severe chronic plaque psoriasis.	NCT02173301
COLBY PHARMACEUTICALS: OT-551	Dry Eye Macular Degeneration	Phase 2 (completed)	Tempol hydroxylamine prodrug. Positive results in safety and tolerability. OT-551 reduced vision loss and conserved visual acuity.	OMEGA/ NCT00485394
VTV THERAPEUTICS: HPP971	Immunological Disorders, Bone, Eye, Lung, Blood diseases	Preclinical	Inhibitor of BACH1 that induces expression of HMOX1 in and NRF2-dependent manner.	Ref. ²⁶⁴
V CLINBIO: VCB-101	Multiple Sclerosis	Preclinical	Glycerol conjugate of monomethyl fumarate and eicosapentaenoic acid	https://www.vclinbio.com
V CLINBIO: VCB-102	Psoriasis		Glycerol conjugate of monomethyl fumarate and docosahexaenoic acid	
GlaxoSmithKline: Compound A	COPD	Preclinical	Potent and selective PPI inhibitor of the KEAP1 Kelch/NRF2 interaction in human bronchial epithelial cells and COPD patients-derived lung cells.	Ref. ²⁴⁶
MOCHIDA: TFM-735	Multiple Sclerosis	Preclinical	Potent NRF2 inducer that inhibits inflammatory cytokine production and disease progression in mice with experimental autoimmune encephalomyelitis.	Ref. ²³⁷
CATABASIS: CAT4001	FRDA, ALS	Preclinical	Conjugate of MMF and docosahexaenoic acid.	https://www.catabasis.com/CATB-2017-IARC-Poster-Reilly.pdf
C4X DISCOVERY: ML334 and derivatives	ND, T2DM, COPD	Preclinical	PPI inhibitors identified through a high-throughput screen using a fluorescence polarization assay.	Ref. ²⁴⁷
KEAPSTONE THERAPEUTICS: KEAP1 inhibitors	PD, ALS	Preclinical	Chemical series of KEAP1 inhibitors developed at the Sheffield Institute for Translational Neuroscience.	https://www.keapstone.com
DAIICHI SANKYO CO: RS9	Retinovascular disease	Preclinical	NRF2 activator derived from products of microbial transformation.	Ref. ³¹⁰
ACLIPSE THERAPEUTICS: M102	ALS and other ND	Preclinical	Selective activator of NRF2 and HSF1 showing disease modifying effects in ALS.	https://www.aclipsetherapeutics.com

Table 2. Half-lives and functions of representative proteins whose genes are transcriptionally regulated by NRF2.

Gene	Product	Function	Half-life (h)	Ref.
<i>Biotransformation and detoxification</i>				
<i>AHR</i>	Aryl Hydrocarbon Receptor	Regulation of xenobiotic detoxifying genes	7.7-9.7	311
<i>CYP1B1</i>	Cytochrome P450, B1	Xenobiotic detoxification	16.57 ± 3.00	312
<i>UGT1A1</i>	UDP-Glucuronosyl Transferase 1, A1	Detoxification reactions based in glucuronidation of small lipophilic molecules	~10	313
<i>GSTM1</i>	Glutathione S-Transferase, Mu 1	Detoxification of carcinogens, therapeutic drugs, xenobiotics and electrophiles by conjugation with glutathione	44.69 ± 4.56	312
<i>ABCB6</i>	ATP-Binding Cassette, B6	Mitochondrial ABC transporter involved in heme synthesis	~24	314
<i>ABCC1</i>	ATP-binding Cassette, C1	Organic anion ABC transporter. Multi-drug resistance	37.15 ± 8.03	312
<i>CBR1</i>	Carbonyl Reductase 1	Reduction of quinones, prostaglandins, menadione, various xenobiotics.	52.92 ± 15.73	312
<i>EPHX1</i>	Epoxide Hydrolase 1	Detoxification of polycyclic aromatic hydrocarbons	27.79 ± 3.40	312
<i>ALDH3A2</i>	Aldehyde Dehydrogenase 3, A2	Detoxification of aldehydes generated by alcohol metabolism and lipid peroxidation	87.00 ± 17.27	312
<i>ADH7</i>	Alcohol Dehydrogenase, class 4	Detoxification of aliphatic alcohols, hydroxysteroids, and lipid peroxidation products.	47.93 ± 7.31	312
<i>NQO1</i>	NAD(P)H:Quinone Oxidoreductase 1	Reduction of quinones to hydroquinones	59.37 ± 13.54	312
<i>Antioxidant enzymes</i>				
<i>GPX4</i>	Glutathione Peroxidase 4	Detoxification of hydrogen peroxide, organic hydroperoxides, and lipid peroxides	~18	315
<i>GSR1</i>	Glutathione Reductase 1	Conversion of glutathione disulfide (GSSG) to the reduced glutathione (GSH)	43.87 ± 6.41	312
<i>TXN1</i>	Thioredoxin 1	Reduction of other proteins by cysteine thiol-disulfide exchange	53.28 ± 5.43	312
<i>PRDX1</i>	Peroxioredoxin 1	Reduction of hydrogen peroxide and alkyl hydroperoxides.	54.41 ± 3.62	312
<i>SRXN1</i>	Sulfiredoxin 1	Reduction of cysteine-sulfinic acid formed in peroxiredoxins	9.50 ± 1.72	312
<i>Carbohydrate metabolism</i>				
<i>G6PD</i>	Glucose-6-Phosphate Dehydrogenase	1- Production of NADPH though the conversion of glucose 6-phosphate to 6-phosphogluconate in the Pentose Phosphate Pathway	46.33 ± 7.43	312
<i>PGD</i>	6-Phosphogluconate Dehydrogenase	Production of NADPH though the conversion of 6-phosphogluconate to ribulose 5-phosphate in the Pentose Phosphate Pathway	51.26 ± 7.72	312

TALDO1	Transaldolase 1	Interconversion of monosaccharides in the Pentose Phosphate Pathway. It links this pathway to glycolysis.	63.58 ± 3.83	312
TKT	Transketolase 1	Interconversion of monosaccharides in the Pentose Phosphate Pathway. It links this pathway to glycolysis.	81.32 ± 11.44	312
ME1	Malic Enzyme 1	Production of NADPH through oxidative decarboxylation of malate to pyruvate	363.84	315
UGDH	UDP-Glucose Dehydrogenase	Conversion of UDP-glucose to UDP-glucuronate for biosynthesis of glycosaminoglycans	37.24 ± 3.60	312
Lipid metabolism				
ACOT7	Acyl-CoA Thioesterase 7	Long-chain acyl-CoA hydrolase	148.61	315
ACOX1	Acyl-CoA Oxidase 1	Peroxisomal fatty acid beta-oxidation	32.10 ± 4.02	312
SCD2	Stearoyl-CoA Desaturase-2	Synthesis of unsaturated fatty acids.	~3.5	316
Heme and iron metabolism				
HMOX1	Heme Oxygenase 1	Heme catabolism. Release of anti-inflammatory carbon monoxide and antioxidant redox cycling biliverdin	~15	317
BLVRA	Biliverdin Reductase A	ROS detoxification through biliverdin/bilirubin redox cycle	56.05 ± 8.21	312,318
BLVRB	Flavin Reductase	Oxidoreductase of methemoglobin	42.66 ± 8.75	312
FECH	Ferrochelatase	Heme biosynthesis	35	319
FTH1	Ferritin Heavy Chain	Storage of iron in a soluble and nontoxic state	16.63 ± 1.68	312
FTL1	Ferritin Light Chain	Storage of iron in a soluble and nontoxic state	30.74	312
Mediators of inflammation				
PLA2G7	Phospholipase A2, group VII	Degradation of platelet-activating factor	6.14	312
PTGR1	Prostaglandin Reductase 1	Inactivation of leukotriene B ₄	90.68	312
CEBPB	CCAAT/Enhancer-Binding Protein β	Regulation of genes involved in immune and inflammatory responses	8.59	312
Regulation of NRF2				
KEAP1	Kelch-like ECH-Associated Protein 1	Negative feedback for regulation of NRF2 stability	12.7	38
NFE2L2	Nuclear Factor (Erythroid-derived 2)-Like 2, (NRF2)	Master regulator of cellular homeostasis (about 250 genes)	0.33	320,321
Protein degradation				
LAMP2A	Lysosome-associated membrane protein 2	Chaperone mediated autophagy	75–110	322
SQSTM1	Sequestosome 1 (p62)	Macroautophagy	6-24	323
PSMB1	Proteasome subunit beta type-1	Core subunit of the proteasome 20S	133	324

Figure 1. Regulation of NRF2 by KEAP1 and its pharmacological targeting. **A**, Representation of a single particle electron microscopy image of the KEAP1 dimer (grey surface)¹³ with the crystal structures of the BTB domain (red ribbons; PDB Ref 5NLB) and Kelch-repeat domains (green ribbons; PDB Ref 1ZGK)²⁴¹ inserted for illustrative purposes. The reactive cysteines C151 and C613 are shown as blue spheres. The precise positions of the C226, C278, and C288 are not known, but they are all located in the IVR domain of KEAP1. The KEAP1 homodimer binds NRF2 at two motifs with low affinity (29-DLG-31) and high affinity (79-ETGE-82) and targets this transcription factor for ubiquitination and proteasomal degradation. Current strategies to disrupt this interaction include: electrophiles that chemically modify sulfhydryl groups of at least cysteines C151, C273, and C288 in KEAP1; PPI inhibitors that alter the docking of NRF2 to KEAP1. **B**, Electrophiles modify cysteine residues in sensitive proteins. Since KEAP1 is very sensitive to thiol reaction, electrophilic compounds easily induce KEAP1 modification. The window that allows therapeutic KEAP1 modification in the absence of unspecific thiol modifications within other proteins is represented in the pink area.

Figure 2. Expression of KEAP1 and NRF2 messenger RNAs in human brain and blood. A, The mRNAs for *KEAP1* and *NFE2L2* are in the 64th and 98th percentile of abundance of the total transcriptome of the brain. Note that *KEAP1* mRNA levels are lower than *NFE2L2* mRNA levels as would be expected since KEAP1 protein has a slower turnover (**Table 2**). B, Differential distribution of the mRNA for *KEAP1* and *NFE2L2* in human gray matter from mature brain specimens after removal of meninges and blood clots³²⁵. Both transcripts are higher in astrocytes and microglia compared to neurons. For A and B, data were obtained from the Brain-RNAseq database. FPKM, Fragments Per Kilobase Million. KDE, Kernel density estimation; for details see reference³²⁶. These values are calculated using the function ‘density’ of the statistical software package ‘R’ (<http://www.r-project.org/>). C and D, The mRNA expression of *KEAP1* and *NFE2L2* in some hematopoietic cells. The size and color of the dots correlates with the abundance of either *KEAP1* (C) or *NFE2L2* (D) mRNAs. Again, mRNA encoding KEAP1 is less abundant than that for NRF2. The relative expression of *KEAP1* is low in monocytes, neutrophils and lymphocytes and high in the erythroid lineage while *NFE2L2* exhibits the opposite trend. For C and D, data were obtained from the normal hematopoiesis samples of the Bloodspot database³²⁷.

Figure 3. A. Crystal structure of the KEAP1 BTB domain (from PDB Ref 5NLB; residues 49-180); the protein is shown as a dimer in a surface representation (lefthand side blue, righthand side grey) with the visible C151 shown in purple. The electrophilic motif of selected C151-interactive ligands is indicated by a purple circle; B. Crystal structure of the Kelch domain of KEAP1 (from PDB Ref 4XMB; residues 322-609)²⁴¹; the protein is shown in a surface representation and the sub-pockets²⁴⁵ of the protein-protein interface are colored as follows: P1 sub-pocket: red (residues 415, 461, 462, 478, 483, 508); P2: blue (363, 380, 381, 414); P3: yellow (364, 509, 556, 571, 602, 603); P4: green (334, 572, 577), P5: cyan (525, 530, 555). Sub-pocket occupancy for the ligands 1-4 was determined from crystal structures 4XMB (by analogy to the bis-amide analogue), 5FNU, 4L7B and 3VNH respectively.

Figure 4. Modulation of cancer risk according to status of NRF2. Low NRF2 levels result from aging decline, functional haplotypes of single nucleotide polymorphisms in the *NFE2L2* gene promoter and by microRNA regulation. At least three factors contribute to increased risk of cancer initiation: uncontrolled redox signaling, increased exposure to oxidant genotoxicity, and dampened detoxification of environmental carcinogens. By contrast, over-activation of NRF2 by somatic mutations in *NFE2L2* or *KEAP1* or by collaboration with the indicated oncogenic signaling pathways increase NRF2 levels and promote carcinogenesis because these cells have a selective advantage to survive high ROS levels as well as to resist chemo- and radiotherapy. Pharmacological activation of NRF2 for therapy of chronic diseases should not exceed the safe therapeutic window. At this time, there is no evidence that pharmacological agents can produce the persistent and strong effects equivalent to those of somatic mutations in *NFE2L2* and *KEAP1* but based on preclinical proof-of-concept studies dosing of specific NRF2 activators should be very carefully monitored in order to assess potential cancer risk.