The multifaceted role of Nrf2 in mitochondrial function

Kira M. Holmström1,2, Rumen V. Kostov3 and Albena T. Dinkova-Kostova3,4,5

Abstract
The transcription factor nuclear factor erythroid 2 p45-related factor 2 (Nrf2) is the master regulator of the cellular redox homeostasis. Nrf2 target genes comprise of a large network of antioxidant enzymes, proteins involved in xenobiotic detoxification, repair and removal of damaged proteins, inhibition of inflammation, as well as other transcription factors. In recent years it has emerged that as part of its role as a regulator of cytoprotective gene expression, Nrf2 impacts mitochondrial function. Increased Nrf2 activity defends against mitochondrial toxins. Reduced glutathione, the principal small molecule antioxidant in the mammalian cell and a product of several of the downstream target genes of Nrf2, counterbalances mitochondrial ROS production. The function of Nrf2 is suppressed in mitochondria-related disorders, such as Parkinson’s disease and Friedrich’s ataxia. Studies using isolated mitochondria and cultured cells have demonstrated that Nrf2 deficiency leads to impaired mitochondrial fatty acid oxidation, respiration and ATP production. Small molecule activators of Nrf2 support mitochondrial integrity by promoting mitophagy and conferring resistance to oxidative stress-mediated permeability transition. Excitingly, recent studies have shown that Nrf2 also affects mitochondrial function in stem cells with implications for stem cell self-renewal, cardiomyocyte regeneration, and neural stem/progenitor cell survival.

Addresses
1 BioMediTech and Tampere University Hospital, University of Tampere, Tampere, Finland
2 Institute of Biotechnology, University of Helsinki, Helsinki, Finland
3 Division of Cancer Research, School of Medicine, University of Dundee, Dundee, Scotland, UK
4 Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA
5 Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Corresponding authors: Holmström, Kira M. (kira.holmstrom@helsinki.fi); Dinkova-Kostova, Albena T. (a.dinkovakostova@dundee.ac.uk)

1. Introduction
The mitochondria are known as the powerhouse of the cell. The process of providing the cell with the bulk of its energy is intimately linked to the production of reactive oxygen species (ROS) during oxidative phosphorylation. In most cells, the mitochondria and NADPH oxidase are the main sources of ROS. Our understanding of the role of ROS within the cell is becoming increasingly complex. The traditional view of ROS simply being a harmful by-product of respiration is giving way to a more intricate picture where the role of ROS as an important signaling molecule is emerging [1,2]. It is however becoming evident that an imbalance in the generation of ROS is a common feature in several disease states, ranging from neurodegeneration and diabetes to cardiovascular disease and cancer [3].

As the master regulator of the cellular redox homeostasis, the cap ‘n’ collar basic leucine zipper (CNC-bZip) transcription factor, nuclear factor erythroid 2 p45-related factor 2 (Nrf2) is well equipped to counterbalance the mitochondrial ROS production and is critical for maintaining the redox balance in the cell [4]. Following exposure to oxidants or electrophiles, Nrf2 accumulates in the nucleus. There, it binds to antioxidant response elements (ARE) in the upstream regulatory regions of genes encoding detoxification and antioxidant enzymes, leading to their enhanced transcription [4–6]. Work from our laboratories and the laboratories of other investigators, has shown that the status of Nrf2 activity affects mitochondrial function, and this has been reviewed [7–10]. The current opinion article briefly summarizes the available experimental evidence and provides an update of the most recent findings in this area.

2. Nrf2 regulation
Under basal conditions, Nrf2 is rapidly turned over, and the function of Nrf2 is primarily regulated by controlling the protein levels of the transcription factor through ubiquitination and proteasomal degradation. There are three known ubiquitin ligase systems that are responsible for Nrf2 degradation (Figure 1). The first discovered and most studied is the Kelch-like ECH-associated protein 1 (Keap1)—Cullin3 (Cul3)/Rbx1 [11–13]. As a negative regulator of Nrf2 [14], Keap1 serves as a substrate adaptor protein for the ubiquitin ligase Cul3/Rbx1. Keap1 binds Nrf2 in the cytoplasm and targets the transcription factor for ubiquitination and proteasomal degradation, maintaining Nrf2 at a low steady state level. Oxidants and electrophiles react with
cysteine sensors within Keap1 [15–17], causing a conformational change [18,19] and the inability of Keap1 to target Nrf2 for degradation [20]. This allows free Nrf2 to accumulate and translocate to the nucleus where it binds to a small Maf protein, activating the expression of its target genes [21,22]. Nrf2 is also subject to degradation following phosphorylation by glycogen synthase kinase 3 (GSK3) via β-transducin repeat-containing protein (β-TrCP), a substrate adaptor for Skp1–Cullin1 (Cul1)/Rbx1-based Cullin–RING E3 ubiquitin ligase; and the E3 ubiquitin ligase Hrd1 which resides in the endoplasmic reticulum (ER). The relative contributions of these systems towards the degradation of Nrf2 depend on the specific conditions. Degradation mediated by Keap1 requires reduced state of its cysteine sensors. Degradation mediated by β-TrCP requires prior phosphorylation of Nrf2 by glycogen synthase kinase 3 (GSK3). Degradation mediated by Hrd1 occurs during ER stress.

Regulation of Nrf2 under homeostatic conditions. Nrf2 is a short-lived protein that is continuously targeted for ubiquitination and proteasomal degradation. Three known ubiquitin ligase systems mediate the degradation of Nrf2: Kelch-like ECH associated protein 1 (Keap1), a substrate adaptor protein for Cullin3 (Cul3)/Rbx1-based Cullin–RING E3 ubiquitin ligase and a cysteine-based sensor for Nrf2 inducers; β-transducin repeat-containing protein (β-TrCP), a substrate adaptor for Skp1–Cullin1 (Cul1)/Rbx1-based Cullin–RING E3 ubiquitin ligase; and the E3 ubiquitin ligase Hrd1 which resides in the endoplasmic reticulum (ER). The relative contributions of these systems towards the degradation of Nrf2 depend on the specific conditions. Degradation mediated by Keap1 requires reduced state of its cysteine sensors. Degradation mediated by β-TrCP requires prior phosphorylation of Nrf2 by glycogen synthase kinase 3 (GSK3). Degradation mediated by Hrd1 occurs during ER stress.

Besides regulation of Nrf2 through its degradation, the function of the transcription factor is also controlled through the spatial distribution of both Nrf2 and Keap1. There are three pools of Nrf2 within the cell. In addition to the predominant cytoplasmic pool, there is a nuclear pool of Nrf2, the redistribution of which is controlled in part by Keap1-mediated degradation and by Nrf2 nuclear import signals and mediators [26]. Nrf2 and Keap1 have also been detected at the outer mitochondrial membrane, tethered to the mitochondrial phosphatase phosphoglycerate mutase family member PGAM5 [27]. The three pools of Nrf2 are highly dynamic and subjected to a further fine-tuned regulation. Thus, it has been reported that the ubiquitin-conjugating enzyme UBE2E3 and its nuclear import receptor importin 11 regulate Nrf2 distribution and activity, by restricting the transcription factor from partitioning to the mitochondria and limiting its repression by nuclear Keap1 [28].
3. Nrf2 and the cellular redox homeostasis
Since its discovery in the mid-1990s [22,29], Nrf2 has been extensively studied. The number of publications on Nrf2 has exceeded 7000, and continues to increase exponentially (http://www.ncbi.nlm.nih.gov/pubmed/?term=nrf2). Nrf2 has been associated with cytoprotective functions in animal models of a range of human disease conditions, and has been implicated in the regulation of over 600 target genes [30]. Nrf2 targets include antioxidant enzymes, proteins involved in xenobiotic metabolism and clearance, protection against heavy metal toxicity, inhibition of inflammation, repair and removal of damaged proteins, as well as other transcription and growth factors [31]. Nrf2 regulates the expression of γ-glutamyl cysteine ligase catalytic (GCLC) and modulatory (GCLM) subunits, glutathione reductase (GR) [21,30,32–35], as well as the four enzymes [i.e. malic enzyme 1 (ME1), isocitrate dehydrogenase 1 (IDH1), glucose-6-phosphate dehydrogenase (G6PD), and 6-phosphogluconate dehydrogenase (6PGD)] that are responsible for the generation of NADPH [36–40], all of which are involved in the biosynthesis and maintenance of reduced glutathione (GSH). In turn, GSH, the principal small molecule antioxidant in the mammalian cell, counterbalances the production of ROS. In more recent years, it has emerged that one of the important functions of Nrf2 is to modulate mitochondrial function, as part of its role as a master regulator of cytoprotective gene expression and the cellular redox homeostasis (Figure 2). The evidence for this is two fold. First, it has been shown that the Nrf2 pathway is upregulated and is involved in protection

![Diagram of mitochondrial function](image.png)

**Figure 2**

**Nrf2 affects mitochondrial function at multiple levels.** Nrf2 activation increases the mitochondrial membrane potential (ΔΨ), the availability of substrates for respiration, and ATP production. Nrf2 positively regulates the levels of NADPH by enhancing the expression of genes encoding glucose-6-phosphate dehydrogenase (G6PD), the enzymes of the pentose phosphate pathway (PPP), malic enzyme 1 (ME1) and isocitrate dehydrogenase 1 (IDH1). In addition to NADPH, ME1 regenerates pyruvate, which can cycle back to the mitochondria. Nrf2 also regulates the levels of GSH by enhancing the expression of genes encoding enzymes involved in its biosynthesis and regeneration from its oxidized form, GSSG, including glutathione reductase (GR). Nrf2 negatively regulates ATP-citrate lyase (ACL), acetyl-CoA carboxylase, fatty acid synthase, and stearoyl CoA desaturase, four critical enzymes involved in fatty acid synthesis (FAS). A decrease in the levels of malonyl-CoA may increase mitochondrial fatty acid oxidation (FAO) by relieving its inhibitory function on carnitine palmitoyltransferase 1 (CPT1), which mediates the transport of long-chain fatty acids into the mitochondria. The red and the blue colors indicate positive and negative regulation by Nrf2, respectively. The presentation of the structure of each respiratory complex is adapted from reference [103]. IMS, mitochondrial intermembrane space.
against mitochondrial toxins. Early work noted that increased Nrf2 activity enhanced resistance to mitochondrial toxins such as the complex I inhibitor rotenone or the complex II inhibitor 3-nitropropionic acid in vitro and in vivo [41–43]. Second, Nrf2 function has been reported to be impaired in mitochondria-related disorders, whereas Nrf2 activation has beneficial effects. For example, the Nrf2 pathway is suppressed in Parkinson’s disease patient-derived olfactory neurosphere cells [44], and Nrf2 activation restores the glutathione levels in these cells [45]. This Nrf2 suppression is especially prominent in Friedrich’s ataxia where Nrf2 activation upon oxidative stress was found to be blocked in patient fibroblasts [46].

4. Nrf2 and mitochondrial homeostasis
In 2008, Lo and colleagues reported that Keap1 associates with PGAM5, establishing a physical link to mitochondria [27]. That same year, an association between Nrf2 and mitochondrial biogenesis was found in cardiomyocytes, where Nrf2 stimulates the biogenesis program through activation of nuclear respiratory factor-1 (NRF-1) [47]. This has since been confirmed in in vivo studies [48]. What we have been interested in establishing is a more direct involvement of Nrf2 in modulating mitochondrial function (recently reviewed in [7,8,10]). We showed that respiration and ATP levels are decreased in cells and mitochondria isolated from Nrf2-knockout (Nrf2-KO) mice, while they are increased in their Keap1-knockout (Keap1-KO) and Keap1-knockdown (Keap1-KD) counterparts [49,50]. Similarly, mitochondrial fatty acid oxidation is impaired in cells and mitochondria isolated from Nrf2-KO mice [51]. This could potentially be the reason for the higher accumulation of triglycerides in the liver upon fasting in these mice [52]. As the activities of the respiratory enzymes are not impaired [49], the decrease in respiration and ATP levels under conditions of Nrf2 deficiency argue that the main limitation is substrate availability.

Mitochondrial integrity is key to overall mitochondrial functionality. Mitophagy has emerged as a way to maintain the organelle integrity, by selectively removing damaged mitochondria [53]. One of the critical players involved in this process is the autophagic adaptor protein sequestosome-1 (SQSTM1/p62) [54]. p62 interacts with the Nrf2-binding site on Keap1, competing with Nrf2 for binding [55,56]. This interaction is further enhanced by phosphorylation [57,58]. Therefore, increased free p62 levels activate the Nrf2 pathway, p62 is also an Nrf2-target gene, thus creating a positive regulatory loop [55,56]. An Nrf2-dependent small-molecule mitophagy inducer (p62-mediated mitophagy inducer – PMI) (Figure 3) was recently discovered. PMI directly disrupts the Nrf2-Keap1 interaction [59] and induces mitophagy independently of dissipation of the mitochondrial membrane potential and the mitochondrial serine/threonine-protein kinase PTEN-induced kinase 1 (PINK1)/Parkin pathway [60].

When mitochondrial integrity is lost beyond repair, the mitochondria can undergo permeability transition to induce cell death [61]. Induction of Nrf2 using the isothiocyanate sulforaphane (Figure 3) [62,63] confers resistance to redox-regulated permeability transition [64], suggesting a further role for the Nrf2 pathway in maintaining mitochondrial integrity.

5. Nrf2, mitochondrial function and neurological conditions
Neurodegenerative disorders are commonly characterized by oxidative stress, mitochondrial dysfunction and protein misfolding, making them ideal targets for Nrf2 activator-mediated therapy (reviewed in [10,65,66]). Nrf2 activation has long been shown to be cytoprotective in both toxicological as well as genetic models of neurodegeneration in vitro and in vivo [67–76]. More recently, we have reported that treatment with the Nrf2 inducers RTA-408, a synthetic cyanoenone triterpenoid, or with the naturally occurring isothiocyanate sulforaphane (Figure 3) restored the mitochondrial membrane potential and protected against dopamine toxicity in primary co-cultures of midbrain neurons and astrocytes isolated from PINK1-KO mice, a model of hereditary early-onset Parkinson’s disease [8]. A wide variety of small molecule activators of the Nrf2 pathway have been established and tested in both in vitro and in vivo models of neurodegenerative diseases, including multiple sclerosis, Parkinson’s, Huntington’s and Alzheimer’s disease (recently reviewed in [77]), showing great promise as potential therapeutic agents. Sulforaphane has shown protective effects in a
| Condition /disease | Species/strain | Damaging agent | Sulforaphane dose | Efficacy endpoints                                                                                                                                                                                                 | References          |
|-------------------|---------------|----------------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Brain injury      | Rat & Sprague Dawley | Controlled cortical impact | 5 mg/kg, i.p., 6 h post-injury | Increase in AQP4 in penumbra; increase in GPx in cortex; increase in GSTx3 and HO-1 in brain microvessels; decrease in loss of tight junction proteins and endothelial cells; decrease in blood-brain barrier permeability and cerebral edema. | Zhao et al. (2005, 2007) [104,105] |
| Brain injury      | Rat & Sprague Dawley | Controlled cortical impact | 5 mg/kg, i.p., 15 min post-injury | Increase in Nrf2, NQO1 and HO-1 in cortex; decreased oxidative damage to lipids, proteins and DNA; decreased brain contusion volume and cortical neuronal death; improved neurologic function. | Hong et al. (2010) [106] |
| Brain injury      | Mouse & C57BL/6 WT and Nrf2−/− | Controlled cortical impact | 5 mg/kg, i.p., 6 h post-injury | Decrease in blood-brain barrier permeability in WT mice; Nrf2−/− mice more sensitive than WT mice & no protection by SFN. | Zhao et al. (2007) [105] |
| Brain injury      | Mouse & ICR WT and Nrf2−/− | Controlled cortical impact | 5 mg/kg, i.p., 15 min post-injury | Increase in Nrf2, NQO1, GSTx1 and HO-1 in cortex; decrease in cerebral edema, blood-brain barrier impairment, cortical apoptosis, and motor deficits. | Hong et al. (2010) [106] |
| Brain injury      | Rat & Wistar | Subarachnoid hemorrhage | 5 mg/kg, i.p., 30 min, 12 h, and 36 h after blood injection | Increase in NQO1 and HO-1, and decrease in MMP9 in spinal cord 4 h after injury; decrease in urinary MIF activity; increase in serotonergic. | Benedict et al. (2012) [111] |
| Spinal cord injury | Mouse & ICR WT and Nrf2−/− | Contusion injury (vascular clip, 10 g) | 5 mg/kg, i.p., 1 h after injury | Decrease in MMP9 and TNFα, vascular permeability changes, inflammatory damage, histologic injury, dying neurons count, and spinal cord edema; enhanced hindlimb locomotor function; Nrf2−/− mice more sensitive than WT mice & no protection by SFN. | Mao et al. (2010, 2011) [108,109] |
| Spinal cord injury | Rat & Fischer | Contusion injury (weight drop, 10 g) | 5 mg/kg, i.p., 15 min after injury, then once a day for 3 days | Decrease in IL-1β, TNFα, iNOS phosphorylation, and contusion volume; improvement in coordination. | Wang et al. (2012) [110] |
| Spinal cord injury | Rat & Sprague-Dawley | Contusion injury (200 kdyn) | 10 or 50 mg/kg, i.p., 10 min and 72 h after injury | Increase in NQO1 and HO-1, and decrease in MMP9 in spinal cord 4 h after injury; decrease in urinary MIF activity; increase in serotonergic. | Benedict et al. (2012) [111] |
| Condition /disease | Species/strain | Damaging agent | Sulforaphane dose | Efficacy endpoints | References |
|--------------------|---------------|----------------|-------------------|-------------------|------------|
| Stroke             | Rat           | Temporary common carotid/middle cerebral artery occlusion | 5 mg/kg, i.p., 15 min post-ischemia | axons caudal to the lesion; enhanced hindlimb locomotor function | Zhao et al. (2006) [112] |
| Alzheimer’s disease | Mouse ICR    | Aβ(1-40) injection, i.c.v. | 30 mg/kg/day, i.p., from day –1 to day 4 post-Aβ | Decrease in impairment of working and contextual memory; no effect on amyloidogenesis | Kim et al. (2013) [74] |
| Parkinson’s disease | Mouse C57BL/6 WT and Nrf2<sup>−/−</sup> | MPTP (for 5 consecutive days starting on day 0) | 50 mg/kg, i.p., on day –1 (2 doses, 8 h apart); then daily doses on day 1, 3 and 5 | Increase in NQO1, HO-1, GCLC and GCLM in striatum and ventral midbrain; decrease in loss of dopaminergic neurons, astrogliosis and microgliosis; decrease in pro-inflammatory mediators (IL6 and TNFα); Nrf2<sup>−/−</sup> mice more sensitive than WT mice & no protection by SFN | Rojo et al. (2010), Innamorato et al. (2010), and Jazwa et al. (2011) [70,72,113] |
| Parkinson’s disease | Mouse C57BL6/SJL | MPTP | | Nrf2<sup>−/−</sup> mice more sensitive than WT mice; protection by Nrf2 overexpression or Keap1 downregulation | Chen et al. (2009) and Williamson et al. (2012) [68,69] |
| Parkinson’s disease | Mouse C57BL/6 | 6-Hydroxy-dopamine-induced lesion | 5 mg/kg, i.p., twice a week for 4 weeks starting after lesion induction | Decrease in motor function deficits; decrease in degeneration of dopaminergic neurons and DNA fragmentation; increase in GSH and GR | Morroni et al. (2013) [114] |
| Parkinson’s disease | Mouse C57BL/6 | Rotenone | 50 mg/kg, i.p., every other day before rotenone for 60 days | Increase in NQO1, HO-1 and LC3-II in cortex and striatum compared to rotenone treatment; decrease in rotenone-induced oxidative damage; decrease in loss of dopaminergic neurons; decrease in motor function deficits | Zhou et al. (2016) [115] |
| Huntington’s disease | Rat Wistar | 2,3-Pyridine-dicarboxylic acid (quinolinic acid) | 5 mg/kg, i.p., 24 h and 5 min before intrastriatal infusion of quinolinic acid | Increase in GSH, GR, and GPx; decrease in oxidized proteins, mitochondrial dysfunction, striatal degeneration and circling behavior | Santana-Martínez et al. (2014) and Luis-García et al. (2016) [116,117] |
| Depression          | Mouse Swiss and C57BL/6 WT and Nrf2<sup>−/−</sup> | LPS | 1 mg/kg, i.p., for 7 consecutive days before and the day after LPS | Compared to WT mice, decrease in dopamine and serotonin levels in prefrontal cortex, retraction of astroglial processes, increased microgliosis and depressive phenotype of Nrf2<sup>−/−</sup> mice without LPS; Increase in HO-1, GCLM | Martín-de-Saavedra et al. (2013) [118] |

(continued on next page)
Several Nrf2 activators are undergoing clinical trials; one of them, BG-12 (Tecfidera), has already entered clinical practice. BG-12 is an oral formulation of the Nrf2 inducer dimethyl fumarate (Figure 3), which is being used for the treatment of relapsing — remitting multiple sclerosis in humans [78,79]. Currently, the Nrf2 activator RTA-408 (Figure 3) is being tested for treatment

| Condition/disease | Species/strain | Damaging agent | Sulforaphane dose | Efficacy endpoints | References |
|-------------------|----------------|---------------|-------------------|-------------------|------------|
| Depression        | Mouse \( \delta \) ICR | Acute stress Chronic stress (28 days) | 1, 3, or 10 mg/kg/day, i.p., for 14 days 10 mg/kg/day, i.p., for 14 days starting on day 14 | Reversal of depressive- and anxiety-like behavior | Wu et al. (2016) [119] |
| Depression        | Mouse \( \delta \) C57BL/6 WT and Nrf2\(^{-/-}\) | Repeated social defeat stress for 10 days | 10 mg/kg, i.p., 30 min before defeat stress or 0.1% dietary glucoraphanin | Attenuation of decreased levels in Keap1, Nrf2, BDNF, p-TrkB, and depression-like behavior; Nrf2\(^{-/-}\) mice more sensitive than WT mice | Yao et al. (2016) [120] |
| Multiple sclerosis| Mouse \( \varphi \) C57BL/6 | (MOG\(_{35-55}\) immunization, followed by Pertussis toxin) | 50 mg/kg, i.p., every other day for 22 days | Inhibition of development and severity of experimental autoimmune encephalomyelitis; increase in HO-1 and NQO1, and decrease in oxidative stress in brain; decrease in MMP9, inflammatory infiltration and demyelination in spinal cord; improved distribution of claudin-5 and occluding; preservation of the blood—brain barrier; inhibition of antigen-specific Th17 responses and enhanced IL10 responses | Li et al. (2013) [121] |
| Multiple sclerosis| Mouse \( \delta \) C57BL/6 | MOG33-55 immunization, followed by Pertussis toxin | 10 mg/kg/day, i.p., myrosinase-activated glucoraphanin beginning 1 week before immunization | Decrease in inflammation (NFkB translocation and IL1\(\beta\)) and apoptosis (Bax and caspase 3) in spinal cord; protection against body weight loss | Giacoppo et al. (2013) [122] |

**Abbreviations:** AQP4, aquaporin 4; BDNF, brain-derived neurotrophic factor; GCLC, glutamate cysteine ligase catalytic subunit; GCLM, glutamate cysteine ligase modulatory subunit; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; HO-1, heme oxygenase 1; I\(\kappa\)B\(\alpha\), nuclear factor kappa-light-chain-enhancer of activated B cells inhibitor, \(\alpha\); IL, interleukin; LC3, microtubule-associated protein light chain 3; LPS, lipopolysaccharide; MIF, macrophage inhibitory factor; MMP9, matrix metalloproteinase 9; MOG, myelin oligodendroglial glycoprotein peptide; MPTP, methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; NQO1, NAD(P)H:quinone oxidoreductase 1; SFN, sulforaphane; TNF\(\alpha\), tumor necrosis factor \(\alpha\); p-TrkB, phosphorylated tropomyosin-receptor-kinase B.
of Friedrich’s ataxia (ClinicalTrials.gov, NCT02255435). The potential of GSK3 inhibitors (Tidegusib) in Alzheimer’s disease was explored in a small Phase II clinical trial. Although in this trial no overall statistically significant clinical benefit for the drug was found, it was noted that there was a significant decrease in the levels of β-secretase 1 (BACE1) in cerebrospinal fluid in a subgroup of patients [80].

A recent study reported the ability of sulforaphane to improve social interaction and verbal communication, reversing abnormal behavior in young men with autism spectrum disorder [81]. Interestingly, granulocytes of children with autism exhibit defects in oxidative phosphorylation and reduced gene expression of Nrf2 [82]. In healthy human subjects, metabolic profiling after a dietary intervention with broccoli as a source of glucoraphanin, the precursor of sulforaphane, has indicated enhanced integration of fatty acid oxidation with the activity of the TCA cycle [83]. Taken together, these studies suggest that sulforaphane-mediated Nrf2 activation may lead to improved mitochondrial function and thus contribute to reversal of the behavioral abnormalities in autism.

6. Nrf2 and mitohormesis

An interesting concept that has grown in popularity is the involvement of Nrf2 signaling in hormesis. Hormesis refers to the exposure to low levels of stress such as ROS, which will prime the cell or organism to better handle future insults [84]. Mitohormesis more specifically suggests that the mitochondria might be essential for this process [85]. Nrf2 has been suggested multiple times to have hormetic potential [84,86,87]. This has been extensively discussed in the context of nutritional antioxidants and dietary restriction [88], where it has been shown that Nrf2 is in part responsible for the beneficial effects of dietary restriction through activation of the phase 2 response. SKN-1, the Nrf ortholog in the nematode Caenorhabditis elegans, has been shown to be a longevity factor [89,90]. SKN-1 activation reduces the accumulation of ROS and increases proteasome activity, stress resistance, and lifespan [89,91]. The exact mechanism is not fully understood, but SKN-1 is responsible for mitochondria-associated redox signaling [90], and for coupling proline catabolism with fatty acid oxidation during limited nutrient availability [92].

Most recently, Nrf2 activation was linked to lithium-mediated lifespan extension in Drosophila melanogaster [93]. Lithium inhibits GSK3, and this inhibition stabilizes and activates Nrf2 (Figure 1), thus extending the lifespan of the flies, specifically at low doses. As with any hormetic response, excessive levels of the toxin, and even excessive Nrf2 activation, has detrimental consequences and decreases lifespan. This is in line with the phenotype of the Keap1-KO mice, which die postnatally from hyperkeratosis of the esophagus due to constitutive Nrf2 activation [94], and with the reduced longevity due to prolonged Nrf2 overexpression in transgenic Drosophila melanogaster [95].

7. Emerging role of Nrf2 in mitochondrial function in stem cells

Although not an entirely novel concept, 2016 has seen a surge in high impact publications that have explored the relationship between Nrf2 and mitochondrial function in the context of stem cell biology. Decreased levels of Nrf2 were shown to correlate with the decrease in regenerative capacity of subventricular zone neural stem/progenitor cells (NSPCs) in the rat [96]. Intriguing work by Khacho and colleagues [97] suggests that dynamic changes in the mitochondrial network during neural stem cell development induce ROS-dependent Nrf2-mediated transcriptional activation of cell differentiation. The metabolic reprogramming from oxidative phosphorylation to glycolytic energy production that takes place during the induction of pluripotent stem cells differentiation is also dependent on ROS-mediated Nrf2 activation [98,99]. In the heart, Nrf2 is necessary for neonatal myocardial regeneration after apex resection by activating paired-like homeodomain transcription factor 2 (Pitx2), which then activates antioxidant genes as well as components of the electron transport chain [100].

The age-related decline in the regenerative function of neural stem/progenitor cells has been causally linked to decreased expression of Nrf2 [98]. A recent report found that Nrf2 activity is impaired in the premature aging disorder Hutchinson-Gilford progeria syndrome (HGPS) due to progerin sequestration of Nrf2, leading to subnuclear mislocalization of the transcription factor [101]. Reactivation of the Nrf2 pathway reverses the cellular phenotype, including key phenotypes of the disease, such as reduced viability of mesenchymal stem cells [101] and impaired autophagy [102], while inactivation of the pathway recapitulates some of the aging phenotypes in HGPS. Together, these studies show that Nrf2 is an important player in stem cell biology and cell senescence, and implicate its role in mitochondrial function as a possible mechanistic link.

8. Concluding remarks and future directions

Work from a number of independent laboratories has convincingly demonstrated that the status of Nrf2 activity affects many aspects of mitochondrial physiology, including mitochondrial biogenesis, fatty acid oxidation, respiration, ATP production, redox homoeostasis, as well as the structural integrity and dynamics of this essential organelle. In parallel to recognizing that many human pathological conditions and aging are associated with mitochondrial dysfunction, it is becoming increasingly apparent that this often coincides with
suppressed Nrf2 signaling. Most excitingly, the ability to reactivate Nrf2 by pharmacological agents is a promising strategy for the prevention or treatment of chronic degenerative diseases and for achieving healthy aging. Importantly, pharmacological Nrf2 activators include phytochemicals (e.g. sulforaphane) that are present in plants, such as cruciferous vegetables, which have been an important part of the human diet for centuries, and are largely responsible for the health-promoting effects of plant-rich diets. As both insufficient as well as persistently high Nrf2 activity can have detrimental consequences, it will be critical to understand what is the appropriate “dose” of Nrf2 activity that would restore the balance and correct the pathologic phenotypes.

Acknowledgments

We thank Troy Faithfull for critical reading of the manuscript. We are extremely grateful to Cancer Research UK (C20955/A18644), the Biotechnology and Biological Sciences Research Council (BB/J007498/1), Reata Pharmaceuticals, the Academy of Finland, the European Research Council, Tampere University Hospital Medical Research Fund and Sigrid Juselius Foundation for financial support.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Holmström KM, Finkel T: Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 2014, 15:411–421.

2. Nickel A, Kohlhass M, Maack C: Mitochondrial reactive oxygen species production and elimination. J Mol Cell Cardiol 2014, 73:26–33.

3. Pham-Huy LA, He H, Pham-Huy C: Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008, 4:89–96.

4. Hayes JD, Dinkova-Kostova AT: The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 2014, 39:199–218.

5. Suzuki T, Motohashi H, Yamamoto M: Toward clinical application of the Keap1–Nrf2 pathway. Trends Pharmacol Sci 2013, 34:340–346.

6. Kenaler TW, Wakabayashi N, Biswal S: Cell survival responses to environmental stresses via the Keap1–Nrf2 pathway. Annu Rev Pharmacol Toxicol 2007, 47:89–116.

7. Dinkova-Kostova AT, Abramov AY: The emerging role of Nrf2 in mitochondrial function. Free Radic Biol Med 2015, 88:179–188.

8. Dinkova-Kostova AT, Baird L, Holmström KM, Meyer CJ, Abramov AY: The spatiotemporal regulation of the Keap1–Nrf2 pathway and its importance in cellular bioenergetics. Biochem Soc Trans 2015, 43:602–610.

9. Itoh K, Ye P, Matsumiya T, Tanji K, Ozaki T: Emerging functional cross-talk between the Keap1–Nrf2 system and mitochondria. J Clin Biochem Nutr 2015, 58:91–97.

10. Esteras N, Dinkova-Kostova AT, Abramov AY: Nrf2 activation in the treatment of neurodegenerative diseases: a focus on its role in mitochondrial bioenergetics and function. Biol Chem 2016, 397:383–400.

11. Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M: Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol 2004, 24:10941–10953.

12. Kobayashi A, Kang MI, Okawa H, Ohtsui M, Zenke Y, Chiba T, et al.: Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteosomal degradation of Nrf2. Mol Cell Biol 2004, 24:7130–7139.

13. Cullinan SB, Gordan JD, Jin J, Harper JW, Diehl JA: 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 2004, 24:8477–8486.

14. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al.: Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 1999, 13:76–86.

15. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al.: Direct evidence that sulf-hydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A 2002, 99:11908–11913.

16. McMahon M, Lamont DJ, Beattie KA, Hayes JD: Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. Proc Natl Acad Sci U S A 2010, 107:18838–18843.

17. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, Suh N, et al.: Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. Proc Natl Acad Sci U S A 2005, 102:4584–4589.

18. Dinkova-Kostova AT, Holtzclaw WD, Wakabayashi N: Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein. Biochemistry 2005, 44:6889–6899.

19. Baird L, Lières D, Swift S, Dinkova-Kostova AT: Regulatory flexibility in the Nrf2-mediated stress response is conferred by conformational cycling of the Keap1–Nrf2 protein complex. Proc Natl Acad Sci U S A 2013, 110:15259–15264.

20. Zhang DD, Hannink M: Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 2003, 23:8137–8151.

21. Hirotsu Y, Katsuoka F, Funayama R, Nagashima T, Nishida Y, Nakayama K, et al.: Nrf2–MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res 2012, 40:10228–10239.

22. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al.: An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997, 236:313–322.

23. Rada P, Rojo AI, Chowdhry S, McMahan M, Hayes JD, Cuadrado A: SCFβ-TrCP promotes glycosgen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol Cell Biol 2011, 31:1121–1133.

24. Chowdhry S, Zhang Y, McMahan M, Sutherland C, Cuadrado A, Hayes JD: 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 2013, 32:3765–3781.

25. Wu T, Zhao F, Gao B, Tan C, Yagishita N, Nakajima T, et al.: Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. Genes Dev 2014, 28:708–722.

Discovery of Keap1.

Discovery of Nrf2 in the context of the environmental stress response.
Role of Nrf2 in mitochondrial function Holmström et al. 89

26. Jain AK, Bloom DA, Jaiswal AK: Nuclear import and export signals in control of Nrf2. J Biol Chem 2005, 280: 29158–29168.

27. Lo SC, Hannick M: PGAM5 tethers a ternary complex containing Keap1 and Nrf2 to mitochondria. Exp Cell Res 2008, 314:1789–1803.

Discovery of the Keap1–Nrf2–PGAM5 ternary complex.

28. Pfaffler KS, Pfaffler SM: The ubiquitin-conjugating enzyme *UBE2E3* and its import receptor importin-11 regulate the localization and activity of the antioxidant transcription factor NFR2. Mol Biol Cell 2015, 26:327–338.

Nrf2 regulation by endogenous UBE2E3.

29. Moi P, Chan K, Asnis I, Cao A, Kan YY: 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 1994, 91:9926–9930.

Discovery of Nrf2.

30. Malhotra D, Portales-Casariego E, Singh A, Srivastava S, Arellano D, Hapell C, et al.: Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Sig profiling and network analysis. Nucleic Acids Res 2010, 38:5718–5734.

31. Baird L, Dinkova-Kostova AT: The cytoprotective role of the Keap1–Nrf2 pathway. Arch Toxicol 2011, 85:241–272.

32. Wild AC, Moinova HR, Mulcahy RT: Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J Biol Chem 1999, 274:33627–33636.

33. Wild AC, Mulcahy RT: Regulation of gamma-glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. Free Radic Res 2000, 32:281–301.

34. MacLeod AK, McMahon M, Plummer SM, Higgins LG, Penning TM, Igarashi K, et al.: Characterization of the cancer chemopreventive NFR2-dependent gene battery in human keratinocytes: demonstration that the Keap1–Nrf2 pathway, and not the BACH1–NFR2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. Carcinogenesis 2009, 30:1571–1580.

35. Agymen AS, Chaeckrady R, Shaw PG, Davidson NE, Visvanathan K, Pandey A, et al.: Transcriptomic and proteomic profiling of Keap1 disrupted and sulfonfate-treated human breast epithelial cells reveals common expression profiles. Breast Cancer Res Treat 2012, 132:175–187.

36. Lee JM, Calkins MJ, Chan K, Kan YY, Johnson JA: Identification of the NF-E2-related factor-2-dependent genes confering protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J Biol Chem 2003, 278:12092–12038.

37. Thimulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S: Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 2002, 62: 5196–5203.

38. Mitsubishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, *et al.*: Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell 2012, 22:66–79.

Nrf2 redirects glucose towards the pentose phosphate pathway in rapidly proliferating cells.

39. Singh A, Hapell C, Mann SK, Akhavan-Mensah G, Carrero J, Kumar S, et al.: Transcription factor NFR2 regulates miR-1 and miR-206 to drive tumorigenesis. J Clin Invest 2013, 123: 2921–2934.

40. Wu KC, Cui JY, Klæsens CD: Beneficial role of Nrf2 in regulating NADPH generation and consumption. Toxicol Sci 2011, 123:590–600.

The role of Nrf2 in NADPH generation and consumption.

41. Lee JM, Shih AY, Murphy TH, Johnson JA: NF-E2-related factor-2 mediates neuroprotection against mitochondrial complex I inhibitors and increased concentrations of intracellular calcium in primary cortical neurons. J Biol Chem 2003, 278: 37948–37956.

42. Shih AY, Imbeault S, Barakauskas V, Erb H, Jiang L, Li P, et al.: Induction of the Nrf2-driven antioxidant response confers neuroprotection during mitochondrial stress in vivo. J Biol Chem 2005, 280:22925–22936.

43. Calkins MJ, Jakel RJ, Johnson DA, Chan K, Kan YY, Johnson JA: Protection from mitochondrial complex II inhibition in vitro and in vivo by Nrf2-mediated transcription. Proc Natl Acad Sci U S A 2005, 102:14443–14448.

Nrf2 protects against mitochondrial complex II inhibition.

44. Matigian N, Abrahamsen G, Sutharsan R, Cook AL, Vitale AM, Nouwens A, et al.: Disease-specific, neurosphere-derived cells as models for brain disorders. Dis Model Mech 2010, 3: 785–798.

45. Cook AL, Vitale AM, Ravishankar S, Matigian N, Sutherland GT, Shank J, *et al.*: Nrf2 activation restores disease related metabolic deficiencies in olfactory neurosphere-derived cells from patients with sporadic Parkinson’s disease. PLoS One 2011, 6:e21907.

46. Paupe V, Dassa EP, Goncalves S, Auchere F, Lonn M, Holmgren A, et al.: Impaired nuclear Nrf2 translocation underlines the oxidative stress response in Friedreich ataxia. PLoS One 2009, 4:e4253.

Nrf2 signalling is impaired in Friedreich ataxia.

47. Plantadosi CA, Carraway MS, Babiker A, Sultman HB: Hemoxgenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. Circ Res 2008, 103:1232–1240.

Nrf2 as a regulator of mitochondrial biogenesis.

48. Merry TL, Ristow M: Nuclear factor erythroid-derived 2-like 2 (NFE2L2, Nrf2) mediates exercise-induced mitochondrial biogenesis and antioxidant response in mice. J Physiol 2016, 594:5195–5207.

49. Holmström KM, Baird L, Zhang Y, Hargreaves I, Chalasani A, Land JM, *et al.*: Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. Biol Open 2013, 2:761–770.

Nrf2 regulates mitochondrial function.

50. Kovac S, Angelova PR, Holmström KM, Zhang Y, Dinkova-Kostova AT: Genetic activation of Nrf2 protects against fasting-induced oxidative stress in livers of mice. PLoS One 2013, 8:e59122.

51. Sun N, Youle RJ, Finkel T: The mitochondrial basis of aging. Mol Cell 2016, 61:654–666.

52. Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, *et al.*: PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 2010, 12:19–24.

p62 dependence of PINK1/Parkin-mediated mitophagy.

53. Komatsu M, Kurohara H, Waguri S, Taguchi K, Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Komatsu M, *et al.*: The mitochondrial basis of aging. Mol Cell 2016, 61:654–666.

54. Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, *et al.*: PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 2010, 12:19–24.

p62 activates Nrf2.

55. Jain A, Lamark T, Stjøttem E, Larsen KB, Awuh JA, Overvatn A, *et al.*: PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 2010, 12:19–24.

p62 and Nrf2 engage in a positive feedback regulatory loop.

56. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, *et al.*: Phosphorylation of p62 activates the
90 Oxidative Toxicology

Keap1–Nrf2 pathway during selective autophagy. Mol Cell 2013, 51:618–631.

Phosphorylation of p62 increases its affinity to Keap1.

58. Hancock R, Bertrand HC, Tsujita T, Naz S, El-Bakry A, Laoruchupong J, et al.: Peptide inhibitors of the Keap1–Nrf2 protein–protein interaction. Free Radiac Biol Med 2012, 52: 444–451.

59. Bertrand HC, Schaap M, Baird L, Georgakopoulos ND, Fowkes A, Thiollier C, et al.: Design, synthesis, and evaluation of triazole derivatives that induce Nrf2 dependent gene products and inhibit the Keap1–Nrf2 protein–protein interaction. J Med Chem 2015, 58:7168–7194.

60. East DA, Fagiani F, Crosby J, Georgakopoulos ND, Bertrand H, Schaap M, et al.: PMI: a ΔΨ-independent pharmacological regulator of mitophagy. Chem Biol 2014, 21:1588–1596.

61. Izzo V, Bravo-San Pedro JM, Sica V, Kroemer G, Galluzzi L: Mitochondrial permeability transition: new findings and persisting uncertainties. Trends Cell Biol 2016, 26:655–667.

62. Zhang Y, Talalay P, Cho CG, Posner GH: A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci U S A 1992, 89:2399–2403.

63. Isolation of sulforaphane as the principal inducer of the prototypical Nrf2 target enzyme NQO1 from broccoli.

64. Yang L, Palijyaguru DL, Kensler TW: Frugal chemoprevention: targeting Nrf2 with foods rich in sulforaphane. Semin Oncol 2016, 43:146–153.

65. Greco T, Fiskum G: Brain mitochondria from rats treated with sulforaphane are resistant to redox-regulated permeability transition. J Bioenerg Biomembr 2010, 42:491–497.

66. Yoshi G, Johnson JA: The Nrf2–ARE pathway: a valuable therapeutic target for the treatment of neurodegenerative diseases. Recent Pat CNS Drug Discov 2012, 7:218–229.

67. Johnson DA, Johnson JA: Nrf2-a therapeutic target for the treatment of neurodegenerative diseases. Free Radiac Biol Med 2015, 88:253–267.

68. Chen PC, Vargas MR, Johnson DA, Johnson JA: Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the α-synuclein mutant (AS3T) mouse model. J Neurosci 2012, 32: 17775–17787.

Nrf2 overexpression in astrocytes provides neuroprotection in a mouse model of Parkinson’s disease.

69. Williamson TP, Johnson DA, Johnson JA: Activation of the Nrf2–ARE pathway by siRNA knockdown of Keap1 reduces oxidative stress and provides partial protection from MPTP-mediated neurotoxicity. Neurotoxicology 2012, 33: 272–279.

70. Jazwa A, Rojo AI, Innamorato NG, Hessae M, Fernandez-Ruiz J, Cuadrado A: Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism. Antioxid Redox Signal 2011, 14:2347–2360.

The Nrf2 inducer sulforaphane is neuroprotective in a mouse model of Parkinson’s disease.

71. Lastras-Becker I, Ulusoy A, Innamorato NG, Sahin G, Rabano A, Kirik D, et al.: α-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson’s disease. Hum Mol Genet 2012, 21:3173–3192.

72. Rojo AI, Innamorato NG, Martin-Moreno AM, De Ceballos ML, Yamamoto M, Cuadrado A: Nrf2 regulates mitochondrial dynamics and neuroinflammation in experimental Parkinson’s disease. Glia 2010, 58:588–598.

Nrf2 regulates neuroinflammation in a mouse model of Parkinson’s disease.

73. Abeti R, Uzun E, Renganathan I, Honda T, Pook MA, Giunti P: Targeting lipid peroxidation and mitochondrial imbalance in Friedreich’s ataxia. Pharmacol Res 2015, 99:344–350.

74. Kim HY, Kim HY, Ehrlich HY, Choi SY, Kim DJ, Kim Y: Amelioration of Alzheimer’s disease by neuroprotective effect of sulforaphane in animal model. Amyloid 2013, 20:7–12.

75. Tsvetkov AS, Arrastae M, Barmanda S, Ando DM, Sharma P, Shaby BA, et al.: Proteostasis of polyglutamine varies among neurons and predicts neurodegeneration. Nat Chem Biol 2013, 9:586–592.

76. Quinti L, Casale M, Moniot S, Pais TF, Kaltenbach LS, Pallos J, et al.: SIRT2- and Nrf2-targeting thiazole-containing compound with therapeutic activity in Huntington’s disease models. Cell Chem Biol 2016, 23:849–861.

77. Yang Y, Jiang S, Yan J, Li Y, Xin Z, Lin Y, et al.: An overview of the molecular mechanisms and novel roles of Nrf2 in neurodegenerative disorders. Cytokine Growth Factor Rev 2016, 27:47–57.

78. Kawapec P, Mukrat A, Wisniewska N, Plc A: The effectiveness of dimethyl fumarate monotherapy in the treatment of relapsing-remitting multiple sclerosis: a systematic review and meta-analysis. Curr Neuroparmacol 2014, 12:256–268.

79. Bomprezzi R: Dimethyl fumarate in the treatment of relapsing-remitting multiple sclerosis: an overview. Ther Adv Neurol Disord 2015, 8:20–30.

80. Lovestone S, Boada M, Dubois B, Hull M, Finne JO, Huppertz HJ, et al.: A phase II trial of tidegablis in Alzheimer’s disease. J Alzheimers Dis 2015, 45:75–86.

81. Singh K, Connors SL, Mackin EA, Smith KD, Fahey JW, et al.: Sulforaphane treatment of autism spectrum disorder (ASD). Proc Natl Acad Sci U S A 2014, 111: 15550–15555.

The Nrf2 inducer sulforaphane improves clinical outcomes in autism.

82. Napoli E, Wong S, Hertz-Picciotto I, Giulivi C: Deficits in bioenergetics and impaired immune response in granulocytes from children with autism. Pediatrics 2014, 133:e1405–1410.

83. Arnah CN, Traka MH, Dainty JR, Defernez M, Janssens A, Leung W, et al.: A diet rich in high-glucoraphanin broccoli interacts with genotype to reduce discordance in plasma metabolite profiles by modulating mitochondrial function. Am J Clin Nutr 2013, 98:712–722.

84. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Majo E, et al.: Identification of a new class of thiazole Nrf2 activators with therapeutic potential in models of Huntington’s disease.

85. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Majo E, et al.: Identification of a new class of thiazole Nrf2 activators with therapeutic potential in models of Huntington’s disease.

86. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Majo E, et al.: Identification of a new class of thiazole Nrf2 activators with therapeutic potential in models of Huntington’s disease.

87. Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Majo E, et al.: Identification of a new class of thiazole Nrf2 activators with therapeutic potential in models of Huntington’s disease.
Nrf2 signalling is repressed in premature aging.

Nrf2 signalling is critical for heart repair.

Zhou G, Meng S, Li Y, Ghebre YT, Cooke JP: cell reprogramming.

Mitochondrial dynamics affects stem cell fate decisions by driving a transcriptional program.

Tsakiri EN, Sykiotis GP, Papassideri IS, Terpos E, Meghaizel C, Khacho M, Clark A, Svoboda DS, Azzi J, MacLaurin JG, Corenblum MJ, Ray S, Remley QW, Long M, Harder B, Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, et al.: Reduced Nrf2 expression mediates the decline in neural stem cell function during a critical middle-age period. Aging Cell 2013, 12:802–813.

Corenblum MJ, Ray S, Remley QW, Long M, Harder B, Zhang DD, et al.: Reduced Nrf2 expression mediates the decline in neural stem cell function during a critical middle-age period. Aging Cell 2013, 12:802–813.

Tsakiri EN, Sykiotis GP, Papassideri IS, Terpos E, Dimopoulos MA, Ghebre YT, Cooke JP: cell reprogramming.

Role of Nrf2 in mitochondrial function Holmström et al.