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Model organisms for investigating the functional involvement of NRF2 in non-communicable diseases

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Abbreviations: β-TrCP, β-transducin repeat containing E3 ubiquitin protein ligase; 6-OHDA, 6-hydroxydopamine; Aβ, amyloid beta; AD, Alzheimer's disease; Ang II, angiotensin II; AIF, Apoptosis-inducing factor; ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; AP-1, activating protein 1; ARE, antioxidant response element; AREDS, Age-Related Eye Disease Study; BBB, blood-brain barrier; BDNF, Brain Derived Neurotrophic Factor; BTB, broad complex; CAT, catalase; CBP, cAMP response element-binding protein-binding protein; CDDO-Im, 2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide; CMD, Choline-devoid methionine-deficient; cnc, Cap 'n' colar; CNS, central nervous system; COX2, cyclooxygenase-2; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; CREB, cAMP response element-binding protein; CRISPR, clustered regularly interspaced palindromic repeats; CSDS, chronic social defeat stress; CSF, cerebrospinal fluid; DBZ, tanshinol borneol ester; DEHP, di-2-ethylhexyl phthalate; DGR, double-glycine repeat; DM, Diabetes mellitus; DMD, Duchenne muscular dystrophy; DMF, dimethyl fumarate; DSS, dextran sodium sulfate; DUOX, dual oxygenase; ECM, extracellular matrix; FGF21, fibroblast growth factor 21; FTD, Frontotemporal dementia; FOXO, forkhead box O; FXR, farnesoid X receptor; G6PD, glucose-6-phosphate dehydrogenase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modulatory subunit; GLT-1, glutamate transporter 1; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione (γ-glutamyl-l-cysteinylglycine); GSK-3, glycogen synthase kinase-3; GSSG, glutathione disulfide; GST, glutathione S-transferase; HFD, high fat diet; HIF1α, hypoxia inducible factor α; HPA, hypothalamic-pituitary-adrenal; HRD1, HMG-CoA reductase degradation 1 homolog; H_2O_2, hydrogen peroxide; HO-1, heme oxygenase-1; IDH, isocitrate dehydrogenase; IGF-1, Insulin growth factor 1; Ilps, Insulin-like peptides; IPC, Insulin-producing cells; IIS, Insulin/IGF signaling; IκB, inhibitor of NF-κB; IKK, IkB kinase; IR, ischemia-reperfusion; ISO, isoproterenol; ITT, insulin tolerance test; IVR, intervening region; iNOS, inducible isoform of nitric oxide synthase; KEAP1, kelch-like ECH-associated protein 1; KO, *KO*; KI, knock-in; LAD, left anterior descending; LAMA2-CMD, lAMA2 congenital muscular dystrophy; LGMD, limb girdle muscular dystrophies; LV, left ventricular; LXRα, liver X receptor α; LPS, lipopolysaccharide; MDD, major depressive disorder; ME1, malic enzyme 1; MARCO, macrophage receptor with collagenous structure; mTOR, mammalian target of rapamycin; MCI, mild cognitive impairment; MTLE, mesial temporal lobe epilepsy; MCD, methionine- and choline-deficient; MAOI, monoamine oxidase inhibitor; MIA, maternal immune activation; MPEP, metabotropic glutamate receptor (mGluR)5 antagonist 2-methyl-6-(phenylethynyl)pyridine; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NAC, N-acetyl cysteine; MAFLD, metabolic dysfunction-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NCDs, non communicable diseases; NAD(P)H, nicotinamide adenine dinucleotide phosphate; NRF2, nuclear factor erythroid 2 p45-related factor 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NOD-like receptor family pyrin domain containing 3; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; NQO1, NAD(P)H:quinone oxidoreductase 1; NRF2, nuclear factor (erythroid-derived 2)-like 2; OGD, oxygen and glucose deprivation; PCP, phencyclidine; PD, Parkinson's disease; PGD, phosphogluconate dehydrogenase; PI3K, phosphoinositide 3 kinase; PNDM, permanent neonatal diabetes mellitus; poly(I:C), polyriboinosinic-polyribocytidilic acid; POS, photoreceptor outer segments; PV, parvalbumin; Prx, peroxiredoxin; RAC3, receptor-associated coactivator 3; RAGE, receptor for the advanced glycation end product; RNAi, RNA interference; RNS, reactive nitrogen species; ROS, reactive oxygen species; RPE, retinal pigment epithelium; RXRα, retinoid X receptor alpha; SCNT, somatic cell nuclear transfer; SERCA2a, sarcoplasmic reticulum Ca2+-ATPase; SFN, SFN; SHP, Small heterodimer partner; SKN-1, skinhead-1; SOD, superoxide dismutase; TAC, transverse aortic arch constriction; TALENs, transcription activator-like effector nucleases; TFEA, transcription factor enrichment analysis; TRAMP, transgenic adenocarcinoma of mouse prostate; Trx, thioredoxin; TrxR, thioredoxin reductase; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VPA, valproic acid or Sodium valproate; WDR23, WD repeat-containing protein 23; WT, wild type; ZFNs, zinc-finger nucleases; γ-GCL, γ-glutamylcysteine synthetase; γGS, γ-glutamate cysteine synthetase; γGT, γ-glutamyl transpeptidase.

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ABSTRACT

Non-communicable chronic diseases (NCDs) are most commonly characterized by age-related loss of homeostasis and/or by cumulative exposures to environmental factors, which lead to low-grade sustained generation of reactive oxygen species (ROS), chronic inflammation and metabolic imbalance. Nuclear factor erythroid 2-like 2 (NRF2) is a basic leucine-zipper transcription factor that regulates the cellular redox homeostasis. NRF2 controls the expression of more than 250 human genes that share in their regulatory regions a *cis*-acting enhancer termed the antioxidant response element (ARE). The products of these genes participate in numerous functions including biotransformation and redox homeostasis, lipid and iron metabolism, inflammation, proteostasis, as well as mitochondrial dynamics and energetics. Thus, it is possible that a single pharmacological NRF2 modulator might mitigate the effect of the main hallmarks of NCDs, including oxidative, proteostatic, inflammatory and/or metabolic stress. Research on model organisms has provided tremendous knowledge of the molecular mechanisms by which NRF2 affects NCDs pathogenesis. This review is a comprehensive summary of the most commonly used model organisms of NCDs in which NRF2 has been genetically or pharmacologically modulated, paving the way for drug development to combat NCDs. We discuss the validity and use of these models and identify future challenges.

1. Introduction

Non-communicable diseases (NCDs) are a group of long-lasting and slowly progressive chronic disorders that have become the leading causes of death; they include cancers, cardiovascular diseases, chronic respiratory diseases, neurodegenerative diseases, diabetes, and many others [1]. NCDs are mainly caused by environmental conditions, and very often they are characterized at the molecular level by disrupted homeostasis, oxidative stress, and chronic inflammation [2]. The transcription factor nuclear factor erythroid 2-like 2 (NRF2, encoded by *NFE2L2*), is a master regulator of homeostatic and adaptive responses that continues to emerge as a critical mediator of a wide range of cellular functions [3].

NRF2 was first discovered and cloned in 1994 as a protein that could bind a tandem repeat of the consensus site for the transcription factors activating protein 1 (AP-1) and Nuclear Factor, Erythroid 2 (NF-E2) [4,5]. Studies in NRF2-disrupted mice found a significant decrease in the levels of glutathione-*S*-transferase (GST) and NAD(P)H: quinone oxidoreductase 1 (NQO1) in the intestine and liver compared to wild-type mice following administration of phenolic antioxidants, suggesting that NRF2 governs a distinct set of genes from NF-E2 [4].

NRF2 belongs to the cap 'n' collar (CNC) family of bZIP transcription factors, together with p45 NF-E2, NRF1 and NRF3, and acts through the formation of a heterodimer with one of the small MAF proteins [6,7]. These heterodimers activate the expression of more than 250 genes that contain in their regulatory sequences the antioxidant response element (ARE) [8,9]. Analysis of the co-crystal structures of NRF2-MAFG-ARE complexes show that the CNC motif stabilizes the conformation of Arg residues in the basic region in contact with DNA phosphates [10] (Fig. 1). Notably, NRF2 is a protein comprised of seven NRF2-ECH homology (Neh) domains, each serving a specific function. Neh4 and Neh5 serve as transactivation domains, facilitating the recruitment of cAMP response element-binding protein (CREB)-binding protein (CBP) and/or receptor-associated coactivator 3 (RAC3). Neh7 plays a crucial role in binding to the negative regulator retinoid X receptor alpha (RXRα), while Neh6 is responsible for interacting with β-transducin repeat-containing protein (β-TrCP), a negative regulator. Neh1 is responsible for forming a heterodimer with the small sMAF protein [11].

NRF2 activity is mainly regulated at the protein level through a continuous process of synthesis and degradation. Under normal (non-stressed) conditions, NRF2 abundance is principally controlled by Kelch-like ECH-associated protein 1 (KEAP1), an E3 ubiquitin ligase substrate adaptor that targets NRF2 for ubiquitination and proteasomal degradation [12]. The continual degradation of NRF2 ensures that only a fraction of newly synthesized NRF2 enters the nucleus to control baseline gene expression. The KEAP1 protein contains a bric-a-brac, tramtrack, broad complex (BTB) domain (also known as poxvirus and zinc finger, POZ, domain), an intervening region (IVR) and a Kelch domain known also as double-glycine repeat (DGR) domain (Fig. 2) [13]. Through the DGR domain within each monomer, the KEAP1 homodimer binds to the DLG and ETGE motifs in the Neh2 domain of NRF2 [2,14]. KEAP1 has several cysteine residues in its sequence that are susceptible to modification by electrophiles, resulting in the inactivation of KEAP1 and the stabilization of NRF2 [15]. In addition to KEAP1, at least three other systems regulate NRF2 proteasomal degradation: the β-TrCP- Cullin1/Rbx1 E3 ligase complex [16], the WD repeat-containing protein 23 (WDR23)-DDB1-Cullin4 E3 ligase complex [17], and the E3 ubiquitin ligase HMG-CoA reductase degradation 1 homolog (HRD1) [18].

Overall, our knowledge of NRF2 function has significantly broadened since its discovery, largely due to research involving model organisms. Numerous studies have highlighted the roles of NRF2 in physiology and its dysregulation during the development and progression of several diseases. Herein, we discuss the crucial role that model organisms play in advancing our understanding of NRF2 mechanisms of action in NCDs. Furthermore, we explore the therapeutic potential of NRF2 in the prevention and treatment of multiple NCDs.

2. Target engagement of NRF2 in non-communicable diseases: regulation of redox and inflammatory balance

The cell contains approximately 20,000 protein-coding genes. Some of them play an imperative role in the regulation of cellular processes and, as such, are often described as guardians. For example, p53 and AMPK are usually dubbed guardians of the genome and metabolism, respectively. Similarly, NRF2 is considered a master orchestrator of cellular homeostasis. Mechanistically, direct transactivation of the expression of more than 250 genes related to redox balance, detoxification, inflammation, metabolism and proteostasis by NRF2 rewires cytoprotective signaling to support the maintenance of stable conditions in cells [19]. Considering this cytoprotective activity, NRF2 is a promising molecular target since oxidative damage and inflammation are the molecular signature of NCDs underpinning their development.

A stable redox environment is a prerequisite for the maintenance of cellular homeostasis. It is well established that the overproduction of reactive oxygen species (ROS), reactive nitrogen species (RNS) and the impairment of internal antioxidant defensive mechanisms underlie the development of a variety of health conditions, including diabetes, cancer, and neurodegenerative and cardiovascular disorders. Cells use multiple pathways to catabolise ROS and defend against oxidative stress. They include non-enzymatic antioxidants, and enzymatic systems, prioritising the latter in antioxidant defense capacity [20,21]. The list of genes that encode antioxidant enzymes and proteins involved in the synthesis of non-enzymatic antioxidants that are transactivated by NRF2 is extensive. Just to mention a few, one of the best characterized NRF2 target genes encodes heme oxygenase-1 (HMOX1), an enzyme involved in degrading and detoxifying heme, that is being increasingly recognized as a protein that protects cells via multiple pathways. In addition, NRF2 binds directly to the promoter regions of three major families of enzymes that catabolise superoxide anion and hydrogen peroxide: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Also, glutathione reductase (GSR) and NAD(P)H dehydrogenase quinone 1 (NQO1), the enzymes responsible for regeneration of glutathione and ubiquinone/α-tocopherol, respectively, are targets for NRF2. Other examples are the glutathione synthesis enzymes, glutamate-cysteine ligase catalytic and modifying subunits (GCLC, GCLM), and glutathione synthetase (GSS). The components of another, close to glutathione, reducing agent pathway, which is thioredoxin system, are the NRF2-depedent genes, including thioredoxin (TXN) itself, thioredoxin reductase 1 (TXNRD1), and thioredoxin interacting protein (TXNIP). Finally, four main enzymes that generate NADPH, which provides reductive power for glutathione and thioredoxin redox cycles, namely glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase 1 (IDH1), malic enzyme 1 (ME1), and phosphogluconate dehydrogenase (PGD) are also targets of NRF2.

The fundamental role of the KEAP1-NRF2 pathway in orchestrating redox control in cells was evidenced in numerous experimental studies. Shortly after reporting ARE-dependent inducible regulation of antioxidant enzyme genes by electrophiles and ROS [22,23], studies on peritoneal macrophages isolated from mice lacking NRF2 transcriptional activity evidenced the prime role of NRF2 in transactivation control of oxidative stress-inducible genes [24]. Interestingly, NRF2 is dispensable

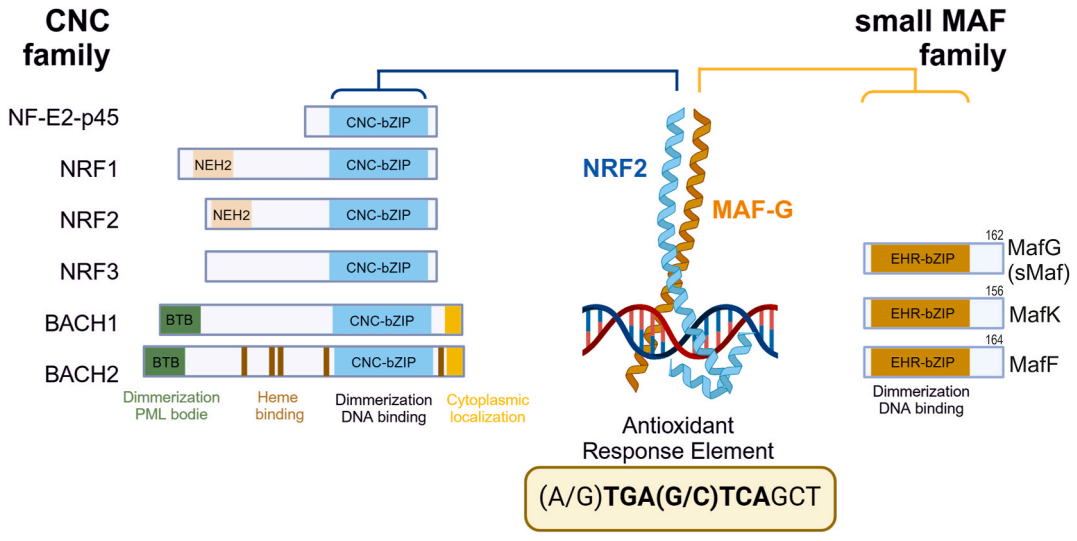

Fig. 1. Members of the small MAF and CNC transcription factor families in mammals. Small Maf proteins (sMafs), comprising MafG (sMaf), MafK, and MafF. The total number of amino acids is depicted to highlight that the molecular weight is different. The CNC family comprises NF-E2p45, NRF1-3, BACH1, and BACH2. Only mammalian family members are shown in this figure. CLS, cytoplasmic localization signal. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

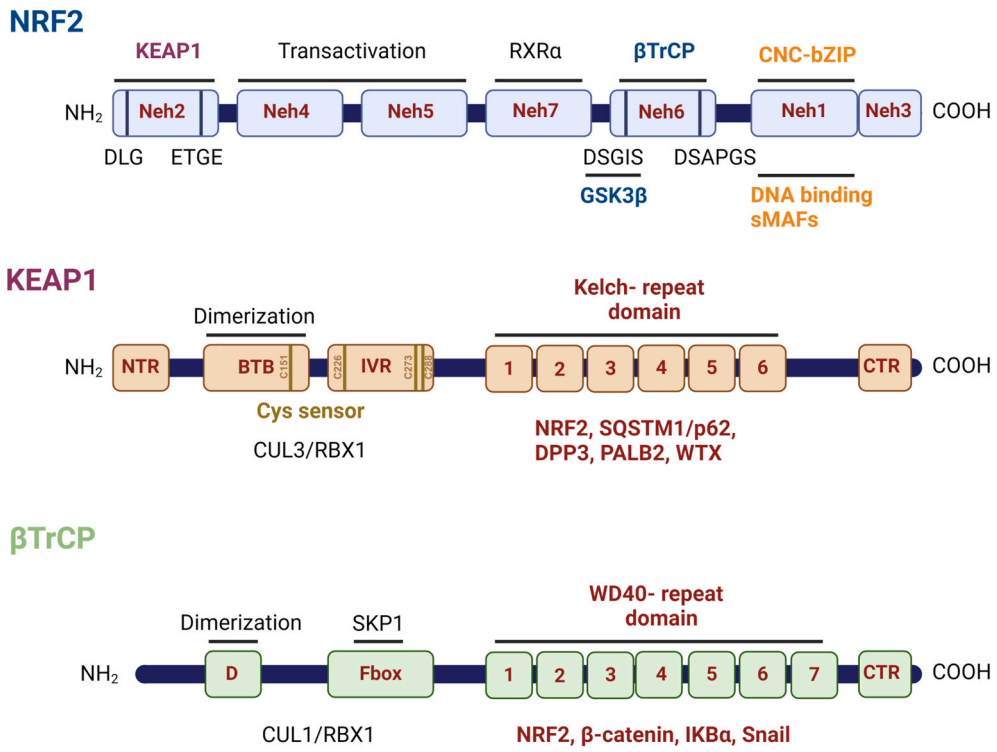

Fig. 2. The architecture of NRF2, KEAP1 and β-TrCP. A, NRF2 contains seven conserved NRF2-ECH homology NRF2-ECH homology (Neh) domains, Neh1-Neh7. Neh1 contains a basic leucine zipper (bZip) motif, where the basic region is responsible for DNA binding and the Zip dimerizes with other binding partners such as sMAFs. Neh2 contains ETGE and DLG motifs, which are required for the interaction with KEAP1 and subsequent KEAP1-mediated proteasomal degradation. Neh3, 4 and 5 domains are transactivation domains of NRF2. Neh6 contains two βTrCP degrons DSGIS and DSAPGS that are responsible for the β-TrCP mediated proteasomal degradation. B, KEAP1 contains five domains, amino terminal region (NTR), a broad complex, tramtrack, bric-a-brac (BTB) domain, an intervening region (IVR), six Kelch domains, and the C-terminal region (CTR). The Kelch domain and CTR mediate the interaction with NRF2, p62, DPP3, WTX, and PALB2 that contains ETGE motifs. The BTB domain homodimerizes with KEAP1 and contributes to the interaction of IVR with Cul3/RBX1 complex. Several functional important cysteine residues (C151, C226, C273 and C278) that sense reactive oxygen species (ROS) and electrophiles and modulate KEAP1-NRF2 interaction. C, βTrCP has three domains, dimerization domain (D) that forms homo- and heterodimers between βTrCP1 and βTrCP2, the F-box that recruits SKP1 for the binding of CUL1/RBX1 complex, and the WD40 repeat domain that binds βTrCP degrons DSGIS and DSAPGS in NRF2. βTrCP, β-transducing repeat-containing protein; CUL3, Cullin3; RBX1, RING-box protein; WD40, WD Repeat protein 40; RXRα, retinoic X receptor alpha; DPP3, dipeptidyl peptidase 3; WTX, Wilms tumor gene on X chromosome; PALB2, Partner and Localizer of BRCA2; GSK3, Glycogen synthase kinase-3; SKP1, S-phase kinase-associated protein-1. Created in BioRender. Rojo, A. (2024) BioRender. com/b33i790.

for normal development as shown in two independently generated strains of NRF2 Knockout (KO) mice [7,25]. Disruption of NRF2 signaling does not influence viability, fertility, erythropoiesis, or growth of mice. Most likely, compensatory/adapting mechanisms related to the regulation of NRF2 target genes by other transcription factors [26] in basal conditions, especially NRF1 as double NRF1/NRF2-KO mice die at early embryonic stages [27], contribute to normal development of these animals. However, experiments based on challenging NRF2-KO mice with electrophiles, oxidative stress inducers, toxic agents, ischemia-reperfusion episodes, hyperoxia, etc., demonstrate the critical role of NRF2 in cellular protection [28]. Striking similarities in combining redox sensing and activation of defensive response pathways are also found in other species, including bacterial OxyR, yeast Yap, *Drosophila* CncC/KEAP1, and zebrafish KEAP1-NRF2 [29,30]. A comparable system also functions in *C. elegans*, but it is not yet clear how SKN-1, repressed by WDR23, is activated by oxidative stress [29].

Although the first paper describing NRF2-KO mice living under normal conditions reported no difference in their health span compared to their wild type (WT) counterparts until the age of 9 months [25], a subsequent study found a decrease in survival in NRF2-KO mice of both sexes, with the predominant effect in female animals. NRF2 deficient mice manifested multiorgan inflammatory infiltrates reminiscent of human systemic lupus erythematosus. The most severe pathophenotype, lupus-like glomerulonephritis, found in the kidney of NRF2-KO mice [31] was also reported in the other murine strain of NRF2 deficiency [32]. These data suggested a critical role of NRF2 in controlling inflammation. The pro-inflammatory phenotype in the absence of NRF2 signaling is also clearly seen in mice subjected to inflammation-related incidents, such as septic shock [33], cigarette smoke-induced emphysema [34], and limb ischemia [35]. The complexity of molecular mechanisms of NRF2-dependent resolution of inflammation has been comprehensively described [19]. It includes direct transactivation of anti-inflammatory gene transcription and repression of pro-inflammatory gene expression, modulation of redox-based inflammation, and multilevel crosstalk of NRF2 with the main pro-inflammatory transcription factor nuclear factor-κB (NF-κB) (Fig. 3). The immunomodulatory effects of NRF2 are crucial in the context of clinical perspective. The anti-inflammatory activity of the NRF2 targeting drugs, dimethyl fumarate and omaveloxolone, currently used for the treatment of multiple sclerosis and Friedrich ataxia, respectively, was one of the rationales for undertaking clinical trials and the final approval of these substances [36,37].

The mechanisms of oxidative stress and inflammation are inextricably linked and may create a snowballing effect that underlies NCD development. Therefore, experimental studies using animal models to investigate the NRF2-related disease pathomechanisms should address not only the hallmark activity of NRF2, which is counteracting oxidative stress, but also its impact in inflammation.

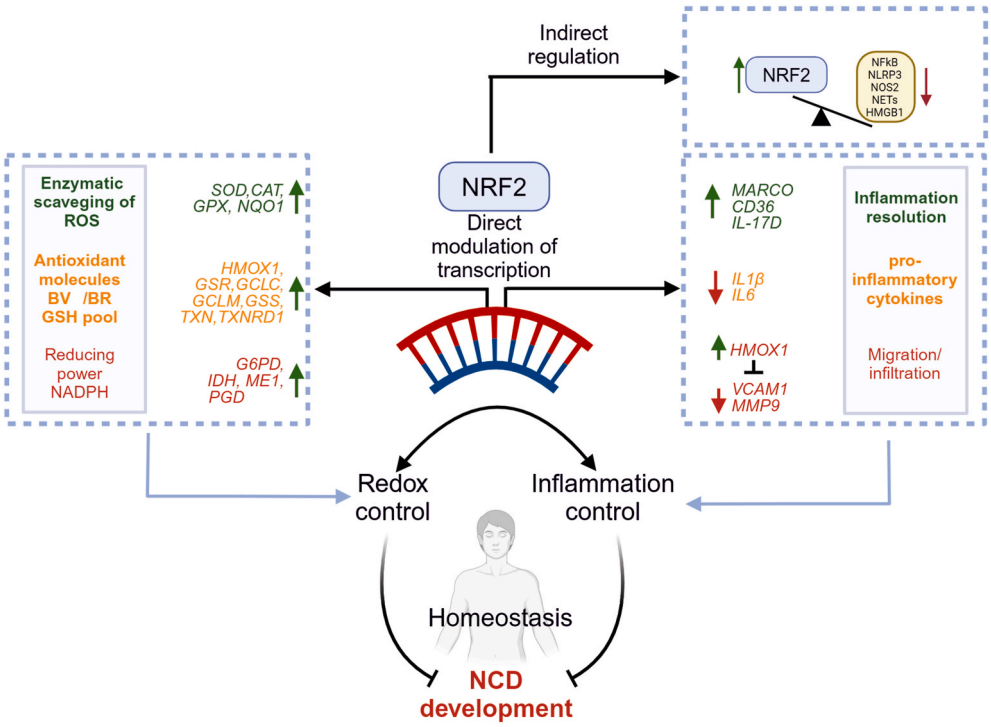

Fig. 3. Maintenance of cellular homeostasis by NRF2-dependent modulation of redox balance and inflammation control. Redox imbalance and inflammation underlie development of non-communicable diseases (NCDs). NRF2 directly transactivates the expression of cytoprotective genes, including anti-oxidative and detoxifying enzymes, which restore proper tissue redox status. NRF2 also facilitates resolution of inflammation, by regulation of the expression of inflammatory genes, its interplay with NF-κB and ROS catabolism. BR, bilirubin; BV, biliverdin; CAT, catalase; G6PD, glucose-6-phosphate dehydrogenase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modulatory subunit; GSH, glutathione; GSS, glutathione synthetase; GSR, glutathione disulfide reductase; Gpx, glutathione peroxidase; GR, glutathione reductase; HMGB1, high mobility group box 1; HMOX1, heme oxygenase-1; IDH1, isocitrate dehydrogenase 1; IL1β, interleukin 1 beta; IL6, interleukin 6; IL17D, interleukin 17D; MARCO, macrophage receptor with collagenous structure; ME1, malic enzyme 1; MMP9, matrix metallopeptidase 9; NCD, non-communicable disease; NETs, neutrophil extracellular traps; NQO1, NAD(P)H quinone dehydrogenase 1; NFKB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; NOS2, nitric oxide synthase 2; PGD, phosphogluconate dehydrogenase; ROS, reactive oxygen species; SOD, superoxide dismutase; TXN, thioredoxin, TXRD1, thioredoxin reductase 1; VCAM1, vascular cell adhesion molecule-1. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

3. Divergence of the KEAP1-NRF2 pathway through evolution

Considering the crucial role of the KEAP1-NRF2 pathway in mediating cytoprotective responses against both intracellular reactive species and extracellular stressors, it is not surprising that the domain architecture of NRF2 is highly conserved across many diverse species of aerobic organisms. Phylogenetic analyses of NRF2 sequences evidenced that its evolution was tightly linked to the rising levels of atmospheric oxygen. In fact, NRF2 is absent in bacteria, archaea, and plants appearing the first functional orthologs in the fungal-metazoan divergence [38], when photosynthetic oxygen was still being absorbed into the oceans. A subsequent significant divergence in NRF2 is seen to occur during the split between fungi and the Metazoa, when free oxygen was resealed to the atmosphere, but with most of this being absorbed by methane oxidation and oxidative weathering of land surfaces. Atmospheric oxygen levels thereafter accumulated giving rise to metazoan success, driving further NRF2 sequence divergence and KEAP1 recruitment for regulation of the oxidative stress response at the division between mammals and non-mammalian vertebrates [39].

In the Fungi taxa, Yap1 protein has been identified as the functional equivalent of NRF2 in yeast [40]. Yap1 operates by binding as a dimer, through its bZIP sequences, to the Yap1 response element (YRE) and activates antioxidant defenses against ROS, heavy metals, and toxic xenobiotics, as extensively reviewed in Ref. [39]. In contrast to mammalian NRF2, which translocates to the nucleus upon the inhibition of KEAP1 mediated repression, fungi rely on a conformational change induced by oxidative stress to retain Yap1 in the nucleus [41]. However, despite their similar mechanisms of action, NRF2 and Yap1 exhibit distinct sequences [39].

The most used species as model organisms to study NCDs comprise invertebrate and vertebrate species (Fig. 4). Regulation of the NRF2 antioxidant response exists in simple invertebrates of the phylum Nematoda, as demonstrated empirically for *Caenorhabditis elegans*. Initially identified for its role in normal pharyngeal development [42], the NRF2 ortholog known as skinhead-1 (SKN-1), offers protection against oxidative stress [43]. There are three *skn-1* splice isoforms (SKN-1a, SKN-1b, and SKN-1c), each with distinct expression patterns and functions. Specifically, SKN-1a is involved in the endoplasmic reticulum stress response [44], SKN-1b regulates lifespan under dietary restriction [45], and SKN-1c regulates expression of antioxidant and detoxification genes [43]. SKN-1c although serving a similar function, has significant differences in structure and regulatory pathways than NRF2 since SKN-1 binds to specific DNA sequences as a monomer [46], due to the absence of the leucine zipper domain crucial for dimerization with small MAFs. Furthermore, the negative regulation of SKN-1 differs from NRF2 in mammals. While bioinformatic analysis [47] has shown that *C. elegans* encodes proteins highly similar to KEAP1, these proteins do not appear to be involved in inhibiting SKN-1. Instead, the inhibition of SKN-1 is accomplished through its interaction with the protein WDR-23 [48]. It has been proposed that homologous KEAP1 proteins of nematodes may have subsequently lost persistence, maybe due to a lack of environmental selective pressure attributed to the hypoxic soil-dwelling lifestyle of worms [39]. Despite these differences in protein regulation, SKN-1 activates target genes like those regulated by vertebrate NRF2 [49].

In Arthropoda taxon, the NRF2 ortholog is the *cnc* gene which was

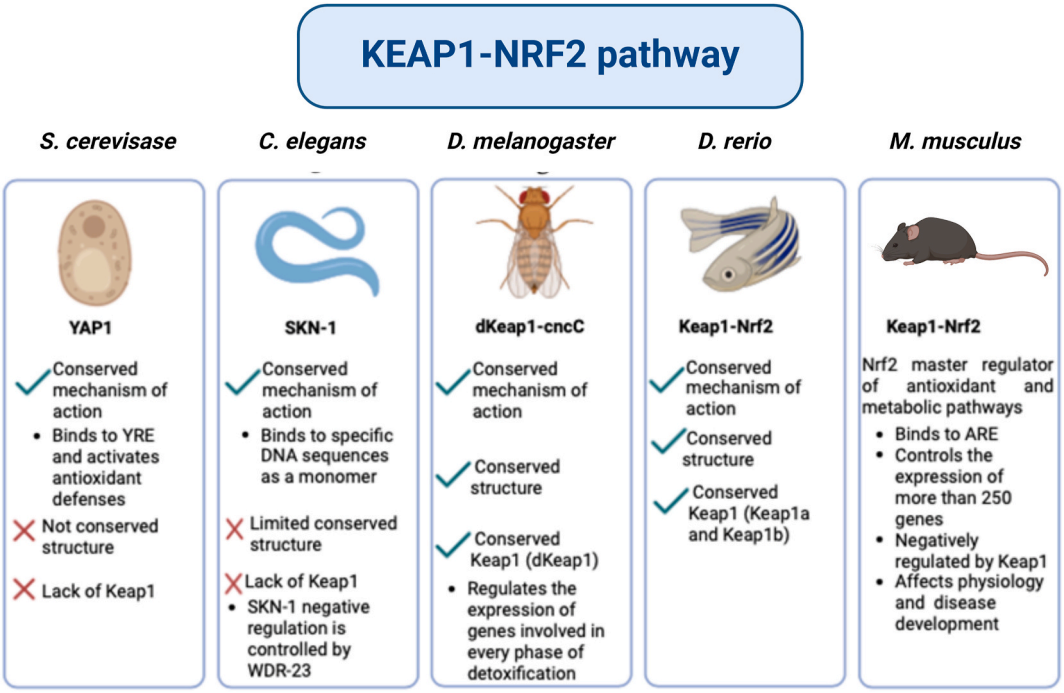

Fig. 4. Summary of the evolutionary conservation of KEAP1-NRF2 pathway. KEAP1-NRF2 exhibits significant homology across species and specifically between *D. melanogaster*, zebrafish (*D. rerio)*, and *M. musculus*. Additionally, in *C. elegans*, SKN-1 functions in a manner like NRF2, but an interesting divergence exists in the regulatory mechanism, as WDR-23 governs SKN-1 activity instead of KEAP1. In yeast, the transcription factor YAP1 is considered as the functional equivalent of NRF2. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

discovered as a protein involved the development of the labral and mandibular structure of larvae in the fruit fly *Drosophila melanogaster* [50]. *NFE2L2* and *cnc* genes shows 60 % similarity encoding for several transcription forms differing in size, N-terminal part structure and expression pattern [51]. At a protein level, three isoforms, cncA, cncB, and cncC, have been described, each possessing a distinct N-terminal region, while sharing a highly homologous C-terminus that encompasses the bZIP structure [52]. CncC splice variant is the one that contains sequences resembling the ETGE and DLG motifs key for their interaction. In fact, cncC activity is regulated by dKEAP1 which exhibits a high degree of homology with its mammalian counterpart [51,53], except that dKEAP1 contains a 156 aa C-terminal tail when removed shows abolished nuclear localization and chromatin-binding [54]. As in mammals, cncC is a known substrate of Slimb (E3 ubiquitin ligase) orthologous to the human β-TrCP being required for normal copper homeostasis in Drosophila [55]. However, in *Drosophila melanogaster*, unlike in vertebrates, cncC regulates the expression of genes involved in phase I and II of the detoxification process [56].

Orthologs of both NRF2 and KEAP1 have been characterized in bony fish taxon being experimentally described in *Danio rerio*, also known as zebrafish. Specifically, zebrafish expresses six nrf genes (*nfe2*, *nrf1a*, *nrf1b*, *nrf2a*, *nrf2b*, and *nrf3*) [57] and two genes encoding KEAP1 proteins (*keap1a* and *keap1b*) [58]. As in mammals, the inhibition of NRF2 by KEAP1 in zebrafish relies on the interaction between the Neh2 and Kelch domains [58]. Notably, zebrafish NRF2 ortholog has been observed to form dimeric complexes with specific proteins, including small MAFs (both MAFG1 and MAFG2) [59], strongly indicating that vertebrates possess an evolutionarily conserved KEAP1-NRF2 pathway. However, in birds a KEAP1 mutation in the Neoavian ancestor disrupted the repression of NRF2 by KEAP1, leading to constitutive NRF2 activity and decreased oxidative stress suggesting the apparition of a compensatory program in NRF2-target genes [60].

4. Model organisms for investigating non-communicable diseases

Gene-edited animals are defined as animals whose DNA has been modified via the use of genetic engineering methods, such as transgenic (carrying an exogenous gene), KO (disrupting an endogenous gene by gene targeting), and knock-in (inserting a gene sequence at a specific location through gene targeting). In the process of creating gene targeting, several techniques play important roles, including homologous recombination, deletions, or insertions of nucleotides in specific gene locus using engineered nucleases such zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system [61]. Each of the following gene-edited animals discussed below has contributed to the current understanding of NRF2 biology.

4.1. Worm models (animalia nematoda Caenorhabditis elegans)

The roundworm *Caenorhabditis elegans* as a model organism allows valuable insights to be gained into the underlying mechanisms of age-related NCDs and the testing of various interventions that could lead to enhanced health span. Years of research have proven its reliability as an important platform for aging research, due to its short lifespan, relatively high level of genetic homology to human biology and the availability of mutant strains with different phenotypes representing the comorbidities associated with aging such as neurodegenerative diseases, obesity, and metabolic disturbances [62]. For instance, the long-lived strains harboring mutations in evolutionarily conserved genes associated with longevity, such as *daf-2*, which encodes insulin/insulin growth factor 1 (IGF-1)-like receptor, and *age-1*, which corresponds the phosphoinositide 3 kinase (PI3K), also exhibit increased survival in response to stressors and maintain a youthful state throughout their lifespan. The extension of lifespan mediated by *daf-2* strongly correlates with the activity of the DAF-16 transcription factor, which is a functional homolog of the Forkhead box O (FOXO) protein family, as well as SKN-1,

the functional homolog of NRF2 in *C. elegans* [63–66].

In nematodes, SKN-1 with its three isoforms (SKN-1A, -1B and -1C), regulates the conserved defense against oxidative stress that is an integral component of phase two detoxification system and consists of numerous enzymes responsible for scavenging free radicals, neutralizing reactive compounds, and facilitating the production or utilization of glutathione. During the embryonic development SKN-1 is indispensable for the digestive system formation, while in larvae and adult phases, it determines the lifespan duration, regulates energy metabolism and stress resistance [65,67]. Neuronal SKN-1 that is constitutively present in the ASI neurons (assumed as hypothalamus) in *C. elegans* is essential for the dietary-restriction-mediated (*daf-2*-dependent) longevity [68,69]. In stress response, regulation of metabolism, and adaptation to starvation, the activation of SKN-1 is dependent on its p38-mediated nuclear translocation in the intestine [70,71]. The activation of SKN-1 has been linked not only to the downregulation of DAF-2 and the activation of DAF-16 but also to the mammalian target of rapamycin (mTOR) signaling pathway, which responds to the scarcity of amino acid and carbohydrates [72]. The negative regulation of SKN-1/NRF2 in nematodes it is not definitively characterized since a clear KEAP1 ortholog in *C. elegans* has not been identified. However, studies suggest evolutionarily conserved functional similarity between KEAP1 and WDR-23 [17,73,74]. In worms, the CUL4-DDB1 ubiquitin ligase complex has been shown to associate with WDR-23, and together, they suppress the expression of oxidative stress genes through regulation of SKN-1 [48] (Fig. 5).

It is worth mentioning, that one of the primary advantages of *C. elegans* as a model system lies in the relatively simple protocols for genetic modification through CRISPR/Cas9-based approach, RNA interference (RNAi) or the utilization of GFP as a fluorescent reporter for tracking target proteins. Correspondingly, mutant strains of *C. elegans* are available with modification in the *skn-1* alleles or the SKN-1 protein isoform facilitating mechanistic modelling of relevant diseases. Loss-of-function *skn-1* mutants exhibit heightened sensitivity to oxidative stress and a shortened lifespan, while overexpression of *skn-1* contributes to increased resilience to stress and prolonged lifespan. More than 30 commercially available mutant strains with a modification in the *skn-1* gene could be obtained by the Caenorhabditis Genetic Centre (CGC, University of Minnesota, MN, USA) including the QV225 *skn-1* (zj15) with hypomorphic allele [67] or the LD1 that expresses skn-1b/c:GFP in the gut and in the ASI neurons [75]. Specifically, the *skn*-1 (zj15) has been generated through the introduction of a point mutation within an intronic region, resulting in the mis-splicing of a fraction of mRNA, which strongly reduces mRNA levels of the two long *skn-1a/c* variants with a stable fraction of homozygous worms that escape embryonic lethality [67,76]. Solid number of studies have employed the *skn-1* (zu67) mutant strains to investigate the involvement of the SKN-1/NRF2 signaling pathway in mediating protective responses against oxidative stress, and the lifespan extension induced by various phytochemicals, such as epicatechin, curcumin, myricetin or quercetin [77]. The collected data suggest that reduction in insulin/IGF-1-like signaling leads to the MAPK-mediated nuclear accumulation of SKN, hence, highlighting the utility of the loss-of-function mutants of MAPK pathways for studying the involvement of *skn-1* in disease modelling [78,79]. Selective activation through gain-of-function (gf) mutation in *skn-1* allele of a pool of SKN-1 sequestered at the outer mitochondrial membrane, has been shown to initiate a state of starvation even in the presence of abundant food. These *skn-1* (gf) mutants have been characterized with transcriptionally active genes associated with metabolism, adaptation to starvation, aging, and survival [73]. Solid number of studies utilize the use of *skn-1*-modified *C. elegans* strains to investigate the molecular mechanism of numerous NCDs such as Alzheimer's disease (AD), obesity, and age-related chronic inflammation (selected examples are provided in Table 1).

Collectively, the high level of functional homology between SKN-1 in *C. elegans* and NRF2 in humans expose this model organism as reliable platform for mechanistic research targeted at its modulation. Moreover, the good susceptibility to genetic manipulations and the well described models of NCDs such as neurodegenerative and metabolic dysregulation in the nematodes further validate the exploitation of *C. elegans* as valuable research tool.

4.2. *Insect models (animalia arthropoda Drosophila melanogaster)*

Over the years, *Drosophila melanogaster* has emerged as a potent model organism for investigating fundamental biological processes. Due to its fully annotated genome, this fruit fly has been one of the key models of genetic research for over a century [80] and has unveiled fundamental biological processes (physiological and pathophysiological), establishing *Drosophila* as an indispensable model organism in the field of biology [81]. The fruit fly has obvious advantages as a model organism since it has a high rate of reproduction and low husbandry cost

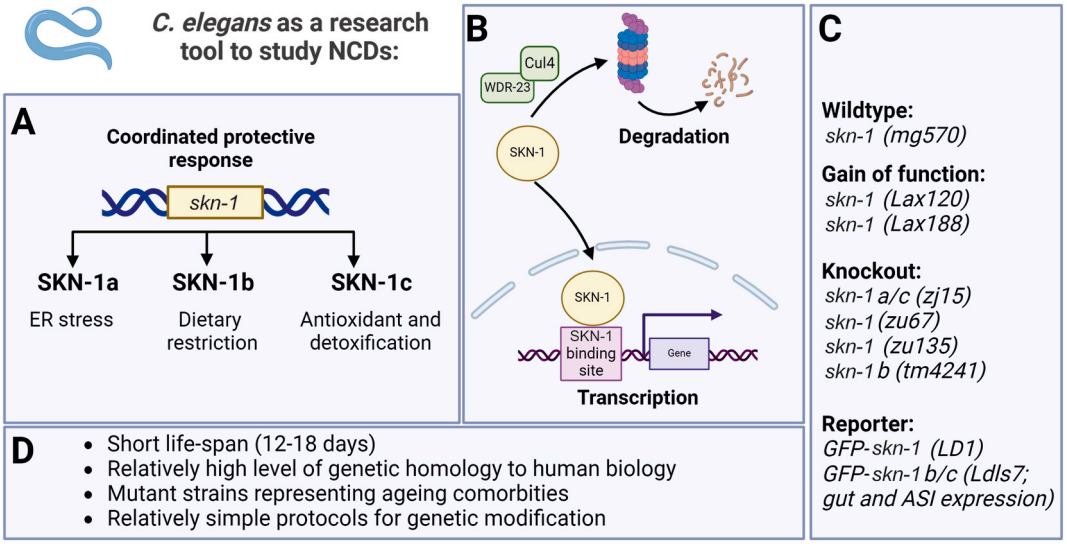

Fig. 5. *C. elegans* as a model organism to study the role of NRF2 in NCDs. A, scheme showing the three isoforms transcribed from the worm *Skn-1* gene to endorse an orchestrated protective response. B, summary of the main regulators of SKN1. C, toolbox of the main *C. elegans* strains employed to analyze the role of Skn-1 in NCDs. D, main advantages of *C. elegans* as a model organism. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

Table 1
Examples of studies that employ *C. elegans* as model species to examine the role of NRF2 in NCDs. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null/interference models for SKN-1 expression (pink), models employing constitutively active SKN-1 (green), and studies using pharmacological activation of SKN-1 (blue).

Study subject	Model	Treatment	Results	PMID
Longevity and ageing	*skn-1(zu135)*. Mutant strain with knock-out skn-1	skn-1 (RNAi), and daf-16 (oe) achieved by three means: zIs356, muEx176 (Pdaf-16: daf-16a:gfp) or muEx227 (Pges-1:daf-16a: gfp, intestine-limited over-expression)	The effects of SKN-1 on oxidative stress and age can be separated, implying that promotion of longevity by SKN-1 can act by mechanisms other than oxidative stress resistance	28612944 [460]
	LD1 (*ldIs7 [skn-1b/c::GFP + rol-6(su1006)]*). Wild-type (normal) C. elegans strain with skn-1 GFP reporter. QV225 (*skn-1(zj15)*). Hypomorphic allele of skn-1 used as knock-out.	Six structurally related flavonoids.	The results showed that chrysin, 6-hydroxyflavone and baicalein prolonged worm lifespan. Baicalein and 6-hydroxyflavone's effects are dependent on the SKN-1 factor	33809299 [79]
	SPC167 *[dvIs19 III; skn-1(lax120) IV]*. SPC167 mutant strain with gst-4p:gfp reporter and with skn-1 (lax120) mutation that gives constitutively activated skn-1	Mulberry anthocyanin extract	Activation of SKN-1, prolonged lifespan and decreased oxidative stress	28713491 [461]
	skn-1 (mg570). Wild type	Grifola frondosa extract	Decreased lipid accumulation	34836223 [78]
Metabolic diseases	skn-1 (lax120); skn-1 (lax188); VC1772: skn-1 (ok2315) IV/nTi [qIs51] (IV; V). Gain-of-function alleles of skn-1	Interference experiments with siRNA against skn-1	Loss of alh-6 accelerates lipid mobilization through SKN-1 mediated FAO genes induction during fasting	25284427 [71]
	ldIs7 [SKN-1b/c: GFP + rol-6 (su1006); ldIs1 [SKN-1b/c: GFP + rol-6 (su1006)]; glp-1 (bn18ts) III; skn-1 (zu135) IV	Ablation of germline stem cells and siRNA experiments against snk-1	SKN-1 plays a direct role in maintaining lipid homeostasis in which it is activated by lipids.	26196144 [462]
	skn-1 (lax120); skn-1 (lax188). Gain-of-function alleles of skn-1	None	These skn-1mutants perceive a state of starvation even in the presence of plentiful food. The aberrant monitoring of cellular nutritional status leads to an altered survival response in which skn-1 mutants transcriptionally activate genes associated with metabolism, adaptation to starvation, aging, and survival	23040073 [73]
	GA1058 skn-1b (tm4241); EU1 skn-1 (zu67), EU31 skn-1 (zu135); skn-1b (tm4241).	Interference experiments with siRNA against skn-1	Neuronal SKN–1B modulates nutritional signalling pathways and mitochondrial networks to control satiety	33661901 [69]
	EU31 *skn-1(zu135)*. The *skn-1 (zu135)* is a mutant strain with knock-out skn-1.	Fuzhuan Tea	Tea reduces AGEs levels by regulating SKN-1	36674689 [463]
Alzheimer`s disease	LD1 (*ldIs7 [skn-1b/c::GFP + rol-6(su1006)]*). Expresses skn-1b/c:GFP in the gut and in the ASI neurons	Fragaria × Ananassa extract. Interference experiments with dsRNA for skn-1	The extract assayed promoted the translocation of the transcription factor SKN-1 to the nucleus, and increased the SOD-3, improve markers of AD, namely oxidative stress and Aβ aggregation in the nematode.	34628121 [464]

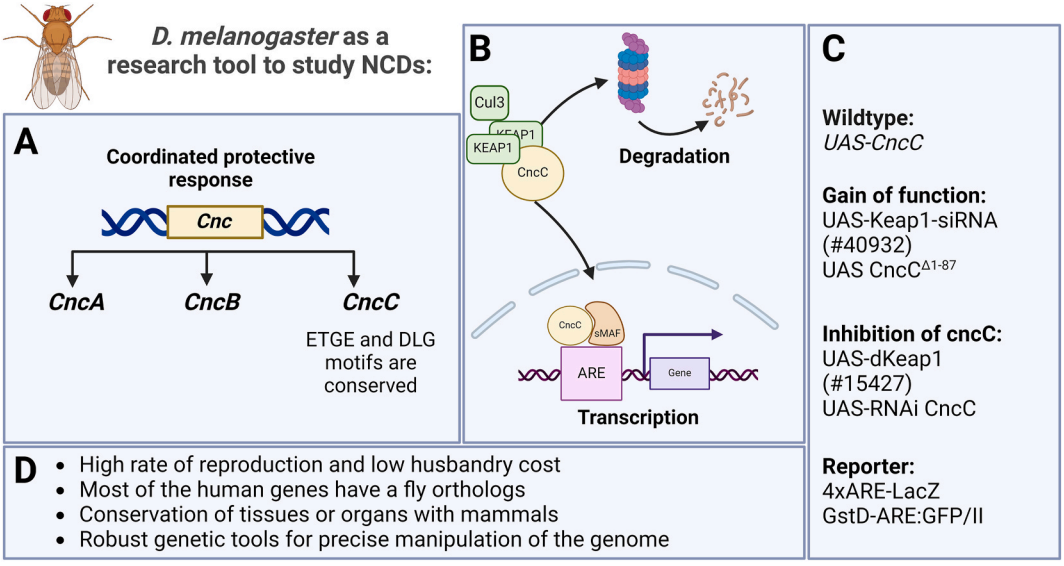

Fig. 6. *D. melanogaster* as a model organism to study the role of NRF2 in NCDs. A, scheme showing the three isoforms transcribed from the fly Cnc gene. B, summary of the main regulators of CncC. C, toolbox of the key stocks of *D. melanogaster* employed to analyze the role of CncC in NCDs. D, main advantages of *D. melanogaster* as a model organism. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

in the laboratory [82]. In addition, there are fewer gene paralogs in *Drosophila* compared to vertebrates, and most of the human disease-related genes have orthologs in flies [83]. Furthermore, *Drosophila* shows structural and physiological conservation with mammals regarding tissues and organs such as the brain, lung, heart, adipose tissue, pancreatic islets, colon, and urinary system, with most of the respective cellular and molecular processes being conserved [84]. The robust genetic tools for precise manipulation of the genome allow excellent spatial and temporal control of gene expression and protein function, including the ability to genetically target different tissues or organs independently, and to manipulate the function of a gene in each tissue [85]. Therefore, *Drosophila* provides a powerful advantage in helping to elucidate mechanisms of NCDs (Fig. 6).

More specifically, *Drosophila* has been attracting significant attention as a model for elucidating molecular mechanisms in cancer. The majority of signaling pathways that regulate cell growth and invasion in mammals perform similar functions in flies, enabling a replication of the biology of tumors [86]. To date, numerous fly models, mimicking various cancer types such as lung, prostate, thyroid, brain, and gastrointestinal cancer [87], have been established to enhance our understanding on tumor initiation and progression and to explore possible therapeutics. Despite anatomical differences with vertebrates, these models have provided insights into various tumor aspects, including the significance of the tumor microenvironment, cancer cachexia, drug resistance and cancer stem cells competition [88]. Single-cell transcriptomics identifies Keap1-NRF2/cncC regulated collective invasion in a *Drosophila* tumor model. Ectopic expression of KEAP1 increased the volume of delaminated follicle cells that showed enhanced invasive behavior with significant changes to the cytoskeleton [89].

Drosophila is the only major invertebrate model organism that contains a beating heart tube and a circulatory system with developmental and functional homologies to the vertebrate heart [90]. The adult fruit fly heart consists of around 80 fully developed cardiomyocytes arranged in two opposing bilateral rows, forming a linear cardiac tube [91]. Differently from mammals, the *Drosophila* heart functions within an open circulatory system, primarily responsible for the transportation of nutrients and signaling molecules rather than oxygen, the latter being circulated through the tracheal system [92,93]. Despite these differences, heart development exhibits remarkable conservation from flies to humans. Not only gene regulatory pathways are conserved, but there is also increasing evidence that mutations in genes linked to cardiac diseases result in fly heart phenotypes that recapitulate clinical observations in humans [94]. These *Drosophila* models have helped in elucidating pathways associated with various cardiac conditions, including arrhythmia [95], atrial fibrillation [96], cardiac aging [97], congenital heart diseases [98], cardiomyopathies [88], and drug-induced cardiotoxicity [99]. Among these studies, multiple outcomes provide strong evidence for the pivotal role of NRF2/cncC signaling in maintaining cardiac health [100]. In that perspective, the *Drosophila* heart presents an appealing system for modelling human cardiac diseases.

The fruit fly can also successfully model metabolic diseases such as diabetes [101]. Same as mammals, the regulation of glucose metabolism is achieved mainly via insulin/IGF signaling (IIS). The insect insulin orthologs, called insulin-like peptides (Ilps), are produced and released from insulin-producing cells (IPC) into the haemolymph, and they control circulating trehalose levels (the glucose equivalent in flies) [102]. Fly models are available for both types of diabetes, which has greatly advanced the study of diabetes and its complications. Rulifson et al. demonstrated that complete depletion of IPC can generate type 1 diabetes phenotypes [103]. Later studies by Broughton et al. showed that similar phenotypes can be generated also after partial elimination of IPC [104]. Unlike type 1 diabetes, type 2 diabetes is mainly characterized by insulin resistance. Fruit fly models of type 2 diabetes have been created by feeding flies with high-sugar or high-fat diets, which result in metabolic disorders like those seen in patients with type 2 diabetes [105]. Given the metabolic alterations observed in response to a high-fat diet, including perturbations in insulin signaling, lipogenesis, and gluconeogenesis, these fruit flies have emerged as a valuable model for conducting research on obesity-related phenotypes [105]. NRF2/cncC has been shown to directly modulate IIS and to affect several metabolic parameters such as glucose and lipid metabolism in flies [106–108], and thus, could play an important role in diabetes.

With the increase in the aging population, neurodegenerative diseases pose a huge burden on society. Although simpler than the mammalian brain, the fly possesses a sophisticated central nervous system comprising neurons (~200,000–300,000) and glial cells, surrounded by a blood-brain barrier [109]. In addition, through the modulation of human gene expression in flies, it is possible to simulate simplified versions of human neurologic diseases. This has extended to various neurologic and neurodegenerative conditions, such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington disease (HD), and spinocerebellar ataxias, aiming to delineate cell biological mechanisms [110]. Thus, the use of *Drosophila* has greatly enhanced our comprehension of the molecular mechanism of several neurologic diseases, leading to the identification of important therapeutic targets [111]. Enhancing the expression of NRF2/cncC in *Drosophila* models of PD yields significant improvements in various aspects of neurodegenerative pathology. This includes the mitigation of neurodegenerative symptoms, the induction of mitophagy, enhanced survival of dopaminergic neurons, and an overall amelioration in the locomotor activity of the flies [112,113]. Furthermore, it is worth mentioning that similar favorable outcomes have been observed also in amyotrophic lateral sclerosis (ALS) models [114]. On the other hand, in AD model, although NRF2/cncC up regulation confirmed mild stress resistance and reduced proteome ubiquitination, it could not overcome Aβ -related toxicity and neurodegenerative phenotypes [115]. These studies underscore the complexity of NRF2/cncC role in neurodegeneration, emphasizing the necessity for further investigation (Table 2).

Finally, *Drosophila* can be used as a screening platform for drug discovery. While there are evolutionary differences, *Drosophila*-based phenotypic screens have emerged as a valuable approach for identifying potential drug candidates [92]. Using the fly model is it possible to contribute to drug discovery via target-identification, *in vivo* validation of known drugs and *in vivo* high-throughput compound screening [116]. Furthermore, *Drosophila* can be a valuable choice for exploring multi-therapeutic strategies, as well as for modelling drug resistance and assessing drug toxicity [117]. Hence, flies offer an attractive model for rapid assay readouts and can be implemented to the discovery and the development of new drugs.

4.3. Fish models (animalia chordata Danio rerio)

As a vertebrate model, zebrafish has the same major organs and tissues as humans their muscle, blood, kidney, and eyes share many features with the human systems. Zebrafish can be grown easily, being an important model in developmental biology because of its reasonably fast generation time and the fact that it has transparent embryos that develop outside of the uterus. In addition, the zebrafish genome has been fully sequenced and shows a similar genetic structure to the human genome.

The KEAP1-NRF2 pathway is conserved in zebrafish, first studies demonstrated an ETGE motif within the Neh2 domain of NRF2 by reverse two-hybrid screening and found to be indispensable for its regulation [118]. Moreover, KEAP1 responded to multiple classes of electrophilic compounds using the "cysteine code" similarly to mammalian [119,120]. An advantage of the zebrafish model is that it often contains duplicate copies of genes that are present as only single copies in mammals thus allowing for additional insight into the multiple functions of the human counterpart [121]. In fact, there are two paralogs of the *NFE2L2* gene, *nrf2a/nfe2l2a* and *nrf2b/nfe2l2b*, the latter without the Neh4 transactivation domain, largely due to the loss of exon 3. These

Table 2
Examples of studies using *D melanogaster* as model organism to examine the role of NRF2 in NCDs. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null/interference models for CncC expression (pink), models employing constitutively active CncC (green), and studies using pharmacological activation of CncC (blue).

Study subject	Model	Treatment	Results	PMID
Longevity and ageing	UAS CncC, UAS CncC$^{\Delta 1\text{-}87}$ and UAS Keap1RNAi	None	CncC activation enhances health span when moderately activated, but intense and chronic overactivation provokes enhances tissues' responses to stress but reduced longevity due to reprogramming of cellular bioenergetics that resulted in the appearance of Diabetes Type 1-like phenotypes.	30537423 [108]
Metabolic diseases	UAS CncC$^{\Delta 1\text{-}87}$	None	CncC ΔN caused a significant reduction in lipid droplet size and triglyceride (TG) levels in the fat body compared to wild type. We found that CncC ΔN induced several genes related to innate immunity that might influence the regulation of cellular lipid storage	26049108 [107]
	Heteroallelic combination for the insulin receptor and the S6 Kinase//heterozygous for a loss-of-function mutation in Keap1	3 % H_2O_2 or 20 mM paraquat laced in the food.	Locomotion was improved in all conditions when CncC was moderately activated by Keap1 heterozygosity	36781022 [106]
Parkinson's disease	A53T-Syn	CDDO-Me (2-cyano-3,12-dioxoolean-1,9 (11)-dien-28-oic acid methyl ester) 100 nmol/L, 1 μmol/L, 10 μmol/L, and 100 μmol/L	Prolonged lifespan. Improved the locomotive abilities and reduced ROS levels	26008822 [465]
	A53T-Syn//Neuronal overexpression of cncC and selectively in dopaminergic neurons	None	Selective expression of cncC in the dopaminergic neurons effectively protected against the neurodegenerative phenotype of the A53T α-synuclein flies, compared to the flies that expressed cncC in all neurons.	26008822 [465]
	Pink1 RNAi, Park RNAi//Overexpression of cncC	None	Induced mitophagy, rescued neuro-muscular degenerative phenotypes	34218254 [112]
Amyotrophic lateral sclerosis	hSOD1-G85R	Urate (5 mM)	Enhanced survival, attenuated motor impairments, reduced oxidative damage and increased antioxidant defense along with Nrf2/CncC activation	30690059 [114]
Cardiac defects	LamC G449V//UAS-RNAi-cncC	None	Suppressed cardiac defects	29575479 [100]
Cancer	Polarity-gene l [2] gl-knockdown//UAS-dKeap1	None	Increased the volume of delaminated follicle cells that showed enhanced invasive behavior with significant changes to the cytoskeleton.	36321803 [89]

genes are expressed in relatively low amounts and in a developmentally and tissue-specific manner. These two paralogs have undergone sub-function partitioning, for instance during embryonic development *nrf2b* functions as a repressor to regulate constitutive gene expression of *p53*, *Cyclin G1*, and *Hmox1 1*. Moreover, embryos in which *nrf2a* expression had been knocked down were more sensitive to *tert*-butyl hydroperoxide but not tert-butylhydroquinone, whereas knockdown of *nrf2b* did not affect sensitivity of embryos to either chemical. Together, these results demonstrate distinct roles for *nrf2a* and *nrf2b*, consistent with sub-function partitioning, and identify a novel negative regulatory role for nrf2b during development [122].

Evolutionary tracing suggests that the *Keap1* gene was duplicated during whole-genome duplication in fish to give rise to *keap1a* and *keap1b*, which bind to Cul3 strongly and weakly, respectively. *Keap1a* was subsequently lost in reptiles and their descendants, and *Keap1b* evolved into mammalian Keap1. The generation of zebrafish *KO* for *keap1a* or/and *keap1b* genes helps to demonstrate that mammalian *Keap1* is an ortholog of fish *keap1b*, not *keap1a* [123]. Interestingly, it has been proposed that molecular evolution of Keap1 was essential for adaptation of vertebrates to terrestrial life, since specific changes in amino acids within the C3IR domain of KEAP1 weakened its interaction with Cul3. In fact, human cells expressing a form of KEAP1 that is similar to that of ancestral aquatic organisms and that binds more strongly to Cul3 are more sensitive to oxidative stress as a result of weak induction of NRF2 [124]. Once in the nucleus, NRF2 heterodimerizes with sMaf proteins. The homolog genes of MafG and MafK but not MafF has been identified in zebrafish, indicating the former two are conserved among vertebrates. In addition, a novel type of small Maf protein MafT was described. MafT protein bound MARE sequence as a homodimer or heterodimers with zebrafish nrf2a/b [59] (Fig. 7).

There are several loss-off-function models of *nrf2a* that pave the way to the study of NCDs. The homozygous mutant *nrf2*^{fh318}, carrying the R485L mutation, displayed enhanced sensitivity to oxidative stress and electrophiles, especially peroxides, and pretreatment with an Nrf2-activating compound, sulforaphane (SFN), decreased peroxide-induced lethality in the wild type but not *nrf2*^{fh318} mutants, indicating that resistance to oxidative stress is highly dependent on Nrf2 functions. These results reveal an evolutionarily conserved role of vertebrate Nrf2 in protection against oxidative stress. Interestingly, there were no significant differences between wild-type and *nrf2*^{fh318} larvae regarding their sensitivity to superoxide and singlet oxygen generators, suggesting that the importance of Nrf2 in oxidative stress protection varies based on the type of reactive oxygen species [125]. Next, CRISPR/cas9 mediated *KO* of *nrf2a* was shown to behave similarly to this previous *nrf2a* mutant; and exhibited higher mortality and a reduced response to H_2O_2 and acetaminophen [126] or to heavy metals [127]. Novel frameshift mutant line *nrf2a*^{it321} has been generated introducing a STOP codon within the second exon by CRISP/Cas9 technology. The analysis of SFN-induced gstp1.2 showed that the induction observed in wild-type larvae was greatly attenuated to levels comparable to controls in *nrf2a*^{it321} homozygous larvae. It was noted that the induction in *nrf2a*^{fh318} larvae was slightly larger than that in *nrf2a*^{it321} larvae, suggesting that the *nrf2a*^{fh318} line is a knockdown line with residual nrf2 activity [128].

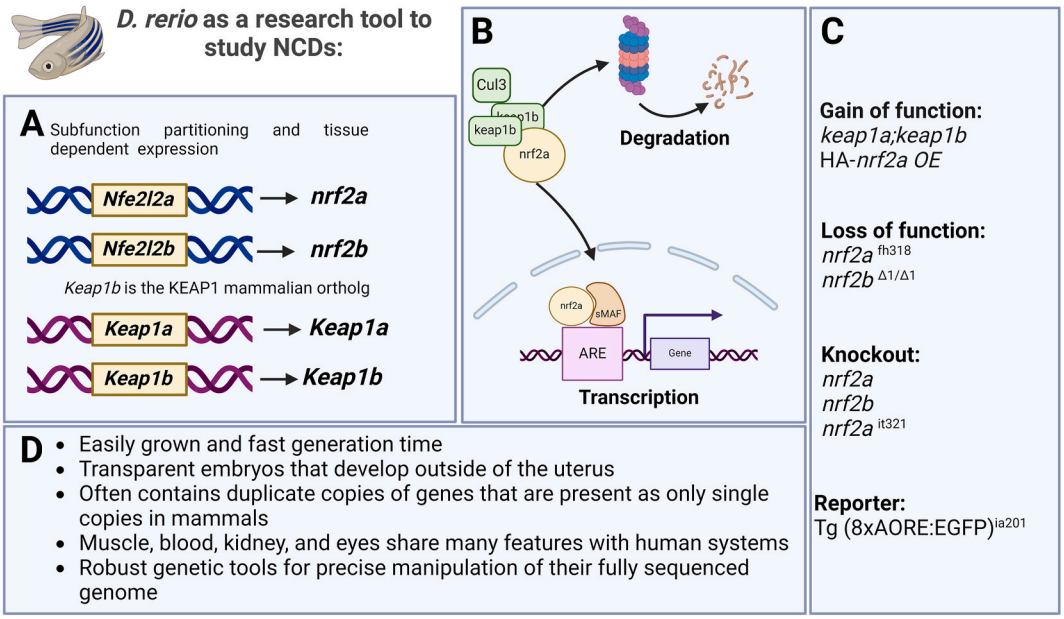

Fig. 7. *D. rerio* as a model organism to study the role of NRF2 in NCDs. A, scheme showing the genomic organization of KEAP1-NRF2 pathway in fish. B, summary of the main regulators of nrf2a. C, toolbox of the key stocks of *D. rerio* employed to analyze the role of nrf2a in NCDs. D, main advantages of *D. rerio* as a model organism. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

In these models is worthy to mention that *nrf2a* and *nrf2b* could also be divergent in their responses to different stimuli as well as interaction partners. In fact, *nrf2*b but not *nrf2*a targeted p53 gene and crossing homozygous *nrf2b* deletion mutant with alanyl-tRNA synthetase (aars1cq71/cq71) mutants resulted in amelioration of defects in neurogenesis [129]. In other studies, *nrf2*^{fh318} homozygous mutants showed compensatory increases in *nfr2b* expression in response to different agents [130], pointing out to a potential compensation for the loss of *nrf2a*.

These diverse mutants and KO of paralogous NRF2 mutants in zebrafish provide a plethora of possibilities to test the evolution and conservation of fish and mammalian KEAP1-NRF2 pathway in physiology and pathology. In fact, *nrf2* overexpression prevented human 4R-tau-GFP and zebrafish 3R-tau-GFP induced neuronal death [131]. Natural products have been tested to reduce MAFLD employing zebrafish model. For example, larval zebrafish submitted to high cholesterol diet exhibited reduced body weight, length and width of hepatitis steatosis when treated with 2,3,5,4′-tetrahydroxystilbence-2-*O*-β-D-glucoside [132] or *p*-hydroxybenzyl alcohol [133]. Although specific activation of NRF2 protein has not been addressed in these studies, *Nfe2l2* and *Hmox1* mRNA levels were increased in treated vs non-treated animals. A summary of the described zebrafish models is shown in Table 3.

With the ever-increasing high throughput omics data, it would be possible to test findings in response to NRF2 inducers and inhibitors by performing *in vivo* experiments in zebrafish and translating these findings to mammalian NRF2 signaling related NCDs. The generation of

Table 3
Summary of the major studies using *D rerio* as model organism to examine the role of NRF2 in NCDs. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are either null expression or loss of function models for *nrf2a* (pink), models employing constitutively active *nrf2a* (green), and studies in which *nrf2a* activation has been detected (blue).

Study subject	Model	Treatment	Results	PMID
Liver and cardiovascular disease	*nrf2a* knockout	hydrogen peroxide, acetaminophen or doxycycline	Showed increased severity and incidence of APAP-induced hepatotoxicity or DOX-induced cardiotoxicity than wild type.	30594530 [466]
Longevity and ageing	*nrf2fh*^{318} (Loss-of-function mutant)	Diethyl maleate, hydrogen peroxide, *tert*-butyl hydroperoxide, paraquat, menadione, rose Bengal, sulforaphane, acetaminophen, methylmercury chloride and/or buthionine sulfoximine.	The nrf2fh^{318} larvae displayed enhanced sensitivity to oxidative stress and electrophiles, especially peroxides, and pretreatment with an Nrf2-activating compound, sulforaphane, decreased peroxide-induced lethality in the wild type but not nrf2fh^{318} mutants	22949501 [125]
	keap1a; keap1b-KO	None	Larval lethality with eating defects. Showed extraordinarily high expression of known *nrf2*-target genes as well as decreased expression of visual cycle genes.	36934645 [467]
Alzheimer's disease	*nrf2a* overexpression	human 4R-tau-GFP and zebrafish 3R-tau-GFP	*nrf2* overexpression prevented human 4R-tau-GFP and zebrafish 3R-tau-GFP induced Neuronal death	26852117 [131]
Metabolic diseases	Wild type	Cholesterol diet and 2,3,5,4′-tetrahydroxystilbence-2-*O*-β-D-glucoside (50 μg/mL) for 1.5 week	Body weight, length and width of hepatitis steatosis of larval zebrafish were decreased in a dose-dependent manner along with higher levels of Nfe2l2, Hmox1 mRNA.	32387861 [468]
	Wild type	Cholesterol diet and P- Hydroxybenzyl Alcohol (20 mg/L) for 1 week)	Improved hepatic lipid metabolism reduced hepatic oxidative stress along with higher levels of *nrf2* and *hmox1* mRNA.	33912056 [133]

novel NRF2/ARE pathway biosensor fish (Tg (8xARE: EGFP) ^{ia201}) will pave the way for high throughput platforms. The transgenic line exhibits a dynamic spatiotemporal expression profile during the early developmental stages and is responsive to known NRF2 pathway modulators including dimethyl fumarate, omaveloxolone, edaravone and CHIR99021 [134].

4.4. Murine models (animalia chordata mammalia Mus musculus)

The use of mice as model organisms to study NCDs is predicated on the genetic and physiological similarities between the species. Despite being bigger than the other model organisms discussed previously, mice are still relatively easily to care for, breed and study. Although issues exist due to differences in immune system performance between mice and humans, the mouse still proves to be the model organism of choice for many studies related to NCDs. The major advantage of using mice is the possibility of modifying their genomes to study specifically the contribution of each gene.

4.4.1. NRF2- and KEAP1-deficient mice as the seeds to unravel NRF2 role in NCDs

The field of NRF2 mouse models has received a big impetus by studies led by the Yamamoto's team, from its beginning in 1997, when Itoh and collaborators generated homozygous NRF2-deficient mice, revealing for the first time that NRF2/sMAF heterodimer mediates the induction of phase II detoxification enzyme genes through the ARE *in vivo* [7]. NRF2-KO mice was generated by a replacement strategy using a targeting vector containing a 4.2-kb segment of DNA with part of exon 4 and all of exon 5 of NRF2 replaced by a 5.5-kb fragment of DNA coding for the bacterial gene LacZ followed by the positive selection neomycin resistance cassette. This replacement vector was engineered to delete the carboxyl 457 amino acid of the NRF2 molecule and to splice in the bacterial reporter gene LacZ. This design would effectively nullify the NRF2 transactivation function since the CNC bZIP regions were deleted. The construct was electroporated into 129X1/SvJ-derived JM-1 embryonic stem (ES) cells. Correctly targeted ES cells were injected into blastocysts and the resulting chimeric mice were used to generate a colony of NRF2-KO mice, which expressed a fusion of the NRF2 N-terminal domains with LacZ [25] (Fig. 8). NRF2-null mice display discolored grey-white incisor teeth, rather than brownish-yellow teeth possessed by wild type mice, due to a loss of iron deposition in the enamel [135].

Likewise, in 2011, the team of Shyam Biswal and Sekhar P. Reddy generated conditional KO mouse with loxP sites flanking exon 5 of the *Nfe2l2 gene* to study the effect of its deficiency in pulmonary Clara cells [136]. Mice carrying the NRF2 "floxed" allele were generated on a C57BL/6 background. The targeting vector inserted LoxP sites flanking the DNA-binding domain of the *Nfe2l2* gene, and a neomycin cassette flanked by flp recombinase target sites for the selection of positive clones. The linearized vector was transfected into C57BL/6J embryonic stem (ES) cells and positive clones were injected into blastocysts. Chimeras with the targeted allele were backcrossed with C57BL/6 mice to generate the NRF2$^{flox/flox}$ and neomycin cassette (Neo)$^{+}$ mice (Fig. 8). These mice allow tissue-specific deletion of NRF2 upon crossing with mice bearing Cre-recombinase under the control of a tissue-specific promoter. In this way, the specific effect of NRF2 deficiency in a cell type can be studied without confounding factors caused by a global loss of NRF2.

The Yamamoto team have used whole-body NRF2-KO mice and their wild-type counterparts to investigate the role of the transcription factor during spaceflight and a 31-day period on the International Space Station. Detailed comparative analyses showed that NRF2 is activated during spaceflight and this activation is important for the adaptation of skeletal muscle [137], kidney [138], and epididymal white adipose tissue [139], the maintenance of blood pressure and bone mineralization [140], as well as for the mitigation of the inflammation, immunosuppression, and thrombotic microangiopathy that occur under these conditions of extreme environmental stress [141].

In work by Yamamoto's team the KEAP1 gene was knocked out in mice through homologous recombination showing that NRF2 constitutively accumulated in nuclei, and NRF2 target genes were constitutively activated [142]. However, the knockout of KEAP1 resulted in post-natal lethality due to hyperkeratosis in the upper digestive tract, which leads to obstructive lesions [142,143]. The body weight gain was retarded, and abnormal metabolic parameters were observed because of malnutrition [144]. Remarkably, this phenotype was rescued by the deletion of NRF2 activity in a KEAP1-null background, indicating that KEAP1-null phenotypes are the consequence of the constitutive activation of NRF2 [142]. The physiological relevance of the cysteine code was evidenced by the generation of different mice lines. The transgenic expression of mutant KEAP1(C273A) and/or KEAP1(C288A) protein in KEAP1 null mice failed to reverse constitutive NRF2 activation, indicating that

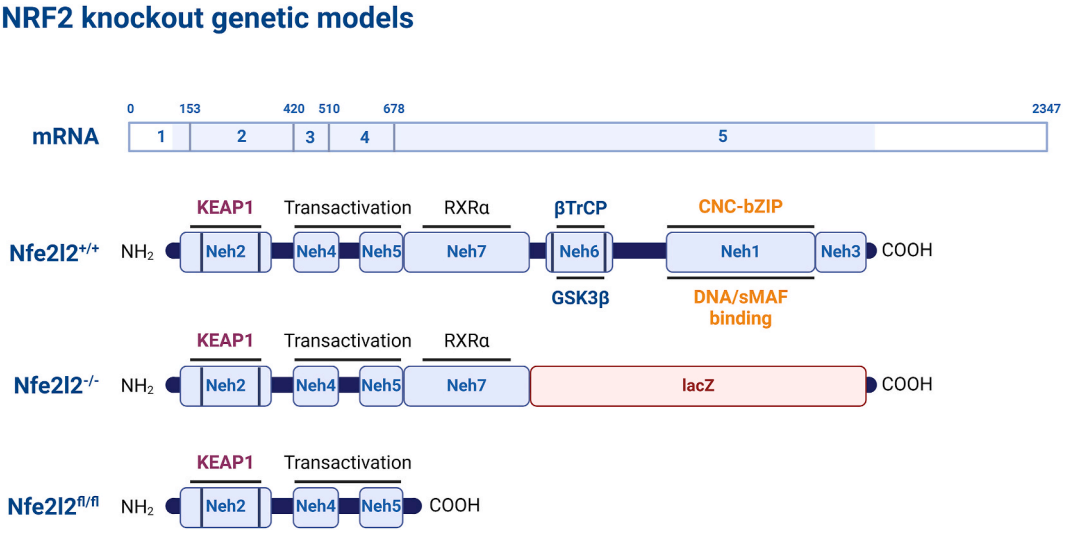

Fig. 8. Domain structure of the different NRF2 knockout mouse models. At the top, the exonic structure of the *Nfe2l2* mature mRNA is presented, indicating different exon-exon junctions. The shaded part corresponds to the coding sequence of the protein. The different domains in the structures below are aligned with the respective exons they are encoded in, considering the specific deletions in the *Nfe2l2* gene each mouse model. Note that both Nfe2l2$^{-/-}$ and Nfe2l2$^{fl/fl}$ are transcriptional knockouts but retain the ability to bind KEAP1 through the Neh2 domain. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

cysteine residues at positions 273 and 288 are essential for KEAP1 to repress NRF2 activity *in vivo*. In contrast, KEAP1(C151S) retained repressor activity and mice expressing this molecule were viable [144] (Fig. 9). The generation of conditional floxed null mice for KEAP1 has been crucial to understand its physiological role. Up to this date, two $Keap1^{fl/fl}$ mice have been generated, that we will term $Keap1^{fl/fl}$ A and $Keap1^{fl/fl}$ B. $Keap1^{fl/fl}$ A mice display a constitutive downregulation of the loxP flanked alleles before Cre-mediated recombination, whereas the $Keap1^{fl/fl}$ B mice express normal levels of KEAP1 before recombination [145–147]. The importance of these models is exemplified by the generation of hepatocyte-specific deletion of the *Keap1* gene by crossing the $Keap1^{fl/fl}$ A1 mice with transgenic mice expressing *Cre* recombinase under the regulation of the *Alb* gene promoter (Alb-Cre mice). This hepatocyte-specific KEAP1 conditional-KO line of mice displayed constitutive NRF2 activation in the liver [148]. $Keap1^{fl/fl}$ B mice were first introduced as a model to study the importance of NRF2 activation in lung tissue against cigarette smoke toxicity. This was achieved by knocking out KEAP1 by crossing these mice with Clara cell-specific Cre-recombinase mice [145].

To address the relative contributions of KEAP1 and β-TrCP-mediated pathways in NRF2 regulation, a new knock-in mouse lines expressing an $NRF2^{SA}$ mutant that harbored two substitution mutations of serine residues interacting with β-TrCP were generated. The homozygous ($NRF2^{SA/SA}$) mice grew normally, with NRF2 levels comparable to those of wild type mice under unstressed conditions. However, when crossed with mice in which KEAP1 was knocked down to two different levels, the $NRF2^{SA/SA}$ mutation induced higher NRF2 activity when the KEAP1 level was strongly reduced, and these mice showed severe growth retardation and hyperplasia and hyperkeratosis in the esophageal epithelium. These results indicate that the β-TrCP- cooperates with the KEAP1-mediated pathway to regulate NRF2 activity, which is apparent when the KEAP1-mediated pathway is profoundly suppressed [149]. NRF2 participates in the formation of a protein complex with Axin1/GSK-3/β-TrCP that is disrupted by WNT-3A, a prototypic canonical WNT ligand, and leads to upregulation of the NRF2 transcriptional signature. The relevance of this novel pathway was assessed in mice with a conditional deletion of Axin1 in the liver, which showed upregulation of the NRF2 signature in hepatocytes and disruption of liver zonation of antioxidant metabolism [150].

The development of these models were crucial steps to understand NRF2 biology and paving the way to the generation of a myriad of engineered mice that will be discussed in the next sections within the context of NCDs (see Fig. 10).

4.4.2. *Mouse models of chronic respiratory diseases*

The lungs are constantly exposed to infectious agents and inhaled irritants, including allergens, cigarette smoke and environmental pollutants. The lung epithelium acts as a physical barrier to these insults, whilst at the same time orchestrating innate immune responses. Epithelial cells sense pathogen and damage-associated molecules and drive the activation of innate effector cells and adaptive immune responses to confer prolonged protection [151]. Abnormal epithelial function and dysregulated immune responses to inhaled insults, in susceptible individuals, promote exaggerated inflammation and extensive lung structural changes that lead to chronic inflammatory lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD) [152].

Examples of studies that have investigated the role of NRF2 in lung diseases are shown in Table 4. In a mouse model of irritant-induced asthma involving short exposure to chlorine gas, NRF2-KO mice were shown to have a more prolonged airway inflammation and hyperresponsiveness, than wild type mice, suggesting a role of NRF2 in resolution of inflammation [153]. NRF2-depleted mice also show greater oxidative stress levels, impaired antioxidant gene activation and a

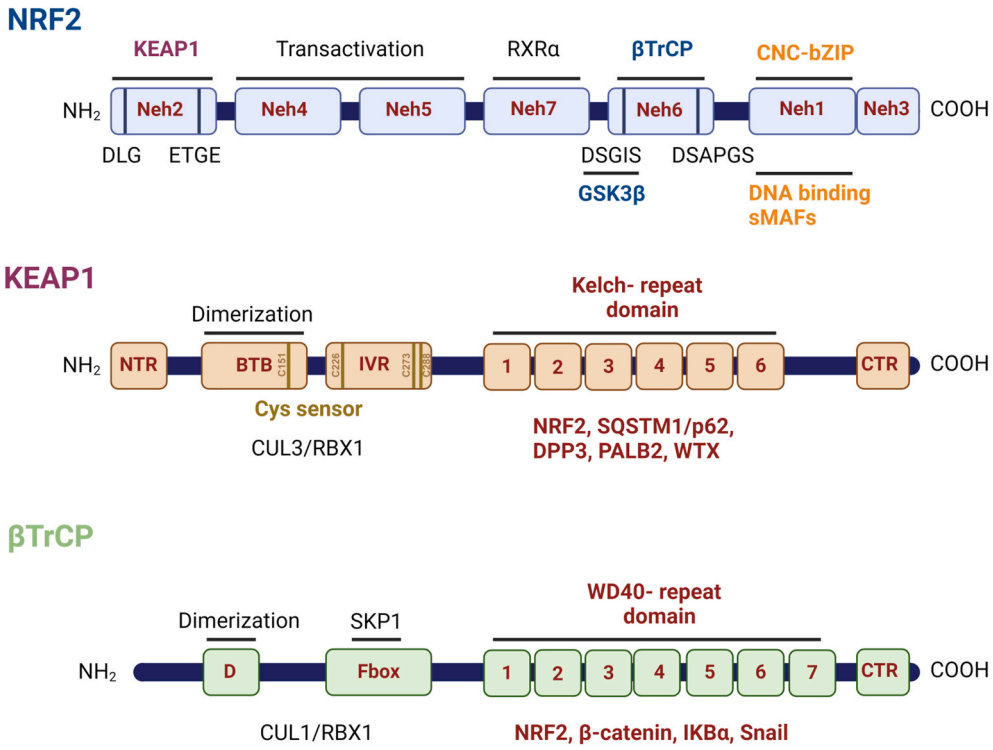

Fig. 9. Domain structure of the different KEAP1 knockout mouse models. At the top, the exonic structure of the *Keap1* mature mRNA is presented, indicating different exon-exon junctions. The shaded part corresponds to the coding sequence of the protein. The different domains in the structures below are aligned with the respective exons they are encoded in, considering the specific deletions in the *Keap1* gene each mouse model. Cysteine redox sensors are labelled in their approximate sites. Note that while in $Keap1^{-/-}$ and $Keap1^{fl/fl}$ B mice the ability to bind CUL3/RBX1 is lost, it is conserved in $Keap1^{fl/fl}$A mice. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

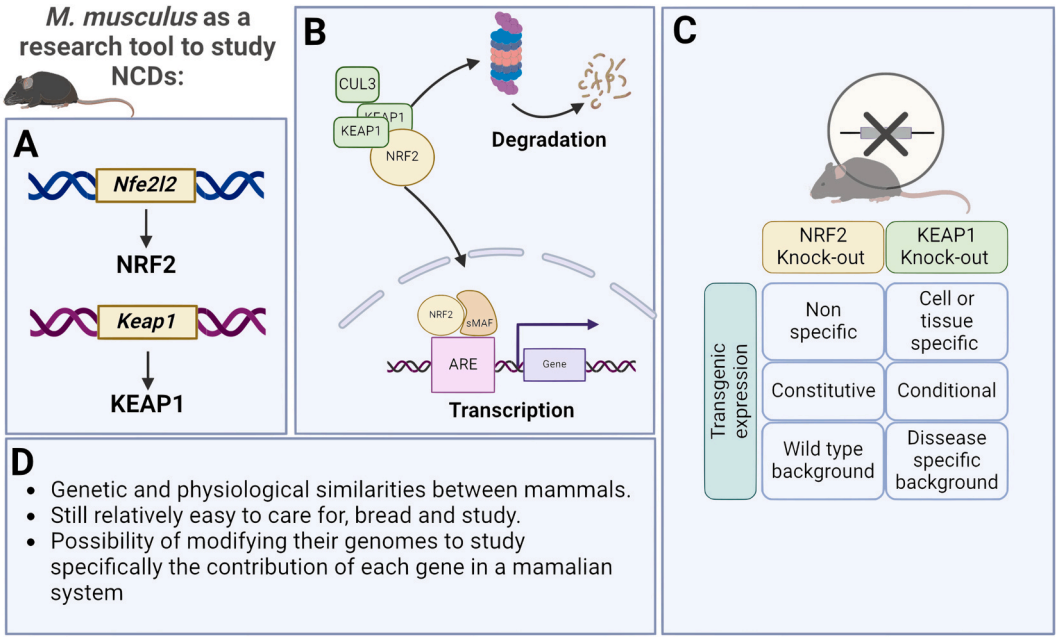

Fig. 10. *M. musculus* as a model organism to study the role of NRF2 in NCDs. A, scheme showing the genomic organization of KEAP1-NRF2 pathway in mammals. B, summary of the main regulators of NRF2. C, toolbox of transgenesis strategies to generate the myriads of mouse models. D, main advantages of *M. musculus* as a model organism. Created in BioRender. Rojo, A. (2024) BioRender.com/b33i790.

greater susceptibility to ovalbumin-induced airway inflammation, hyperresponsiveness and remodeling, indicating a protective effect of NRF2. This protective effect is, at least partly, dependent on the anti-oxidant effects of NRF2 as the antioxidant *N*-acetyl cysteine partly reversed the pathological phenotype in NRF2-null mice [154]. SFN also attenuated airway inflammation and potentiated the anti-inflammatory effects of corticosteroids in cockroach extract-exposed mice [155]. Another study used epithelial cell-specific activation of NRF2, by expressing a non-functional KEAP1 protein, in an ovalbumin-induced mouse model. NRF2 activation reduced oxidative damage of the epithelium, maintaining its barrier function, and inhibited airway mucus secretion, inflammation and hyperresponsiveness [156]. These findings suggest that the protective effect of NRF2 in this model may depend on its effects on the lung epithelium and highlight the potential for pulmonary delivery of NRF2 activators as therapeutic intervention.

Inhalation of cigarette smoke or environmental pollutants may lead to increased severity and poor control of asthma symptoms, and frequent exacerbations [157,158]. This effect is recapitulated using mouse models exposed to a combination of ovalbumin and air pollutants, where the pollutants exaggerate the allergic inflammatory response and lung pathology [159]. Dendritic cells isolated from NRF2-KO mice are more sensitive to particulate matter-induced oxidative stress and inflammatory cytokine release and have a greater ability to induce T2-type cytokine production from naïve CD4 T cells [160]. Adoptive transfer of NRF2-KO mouse dendritic cells into wild type mice increased the adjuvant effect of intranasal ultrafine particulate matter administration on ovalbumin-induced inflammation [161]. Intraperitoneal treatment with SFN improved the sensitivity of mice exposed to ovalbumin and cigarette smoke to the anti-inflammatory effects of corticosteroids. As this effect was only observed in wild type, but not NRF2-KO mice, it is possible that it is mediated by NRF2-dependent mechanisms [162]. The NRF2 activator omaveloxolone reduced lung neutrophilia, and airway hyperresponsiveness and remodeling in a mouse model of asthma exacerbation induced by ovalbumin and ozone exposure [163].

The currently used mouse models have yielded important findings regarding the role of NRF2 in allergic sensitization, development and resolution of inflammation, lung pathology and exacerbations of asthma. Although, the protective effects of NRF2 in these models may be mediated, at least partly, through its antioxidant/cytoprotective function, very little is known regarding other pathways that may confer these effects. More effort is required for elucidating the mechanisms driving the protective effects of NRF2 in these models. Furthermore, new, and more relevant models are required. Asthma mouse models are predominantly allergen-driven and therefore characterized by a T2 inflammatory response. Most of these studies use ovalbumin as an allergen due to its low cost and the very well characterized immune response and lung pathology it induces. Nonetheless, ovalbumin is not relevant to human asthma and thus these models have limited translational potential, compared to other allergens like house dust mite and cockroach extract and pollen. Furthermore, there are currently very few models that represent non-T2 asthma, where oxidative stress may play a key pathogenic role [164], and where NRF2 could be an interesting therapeutic target. The available non-T2 models involve adoptive transfer of Th1 or Th17 cells and are, therefore, of limited translational potential [165,166]. Given the heterogeneity of asthma, mouse models of other known phenotypes and endotypes of asthma are needed.

There is evidence of impaired NRF2-mediated signaling in patients with COPD. Alveolar macrophages and blood mononuclear cells from patients with COPD show reduced NRF2 expression [167]. Moreover, monocyte-derived macrophages from COPD patients show attenuated oxidative stress-induced NRF2 activation, due to increased NRF2 acetylation and degradation [168]. These studies suggest a potential beneficial effect of NRF2 activators as therapeutic interventions for COPD. A randomized control study investigating the effect of oral administration of SFN on patients with COPD, showed no effect on NRF2 target gene expression in alveolar macrophages and bronchial epithelial cells, and no modulation of lung inflammatory and oxidative stress markers or lung function [169]. The effect of more specific NRF2 activators, preferably through the inhalation route, will need to be tested in COPD.

The cigarette smoke (CS) mouse model has been extensively used to study the role of NRF2 in COPD pathogenesis. Specifically, NRF2-KO mice were shown to have increased oxidative stress levels, epithelial cell apoptosis and inflammation, and earlier-onset and more severe emphysema [170]. In a viral-induced exacerbation model, cigarette smoke-exposed NRF2-KO mice exhibited greater inflammatory

Table 4
Summary of the major studies using *M musculus* as model organism to examine the role of NRF2 in lung diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are either null expression models for NRF2 (pink), and studies in which NRF2 has been activated pharmacologically (blue). *Cigarette smoke (CS); cockroach allergen extract (CE); intraperitoneal injection (i.p); N-acetyl cysteine (NAC); lipopolysaccharides (LPS); ovalbumin (OVA); particulate matter (PM); plaque-forming units (PFU); sulforaphane (SFN); ultrafine particles (UFP).*

Study object	Model	Treatment	Results	PMID
Asthma	NRF2-KO vs NRF2-WT	Sensitization on day 0 (20 μg i.p.) and day 14 (100 μg i.p.). Intratracheal instillation of OVA 200 μg on days 24, 26 and 28.	NRF2-KO mice showed more pronounced eosinophil infiltration and mucous production than NRF2-WT	15998787 [154]
	NRF2-KO vs NRF2-WT	Dendritic cells isolated from both genotypes were stimulated with particulate matter	Dendritic cells from NRF2-KO showed greater ROS levels and inflammatory cytokine release in response to particulate matter than NRF2-WT	18802057 [160]
	NRF2-KO vs NRF2-WT	Sensitization on day 0 with intranasal administration of UFP (0.5 μg) and on days 2, 4, 7 and 9 with UFP (0.5 μg) and OVA (10 μg). 2 weeks post-sensitization challenged with aerosolized OVA (1 %; 30mins) for 48hrs	In NRF2-KO mice, UFP had a greater adjuvant effect on OVA-induced allergic inflammation. Adoptive transfer of dendritic cells from NRF2-KO mice to WT mice also led to a greater sensitization by UFP	23595026 [161]
	KEAP1-KO vs KEAP1-WT	Sensitization with i.p administration of OVA (20 μg) on day 0 and day 14 (OVA 100 μg). From day 21, four intratracheal administration of OVA (100 μg) every 48hrs.	Epithelial-specific NRF2 activation led to reduced allergen-induced airway hyperresponsiveness, inflammation, mucus, Th2 cytokine secretion, oxidative stress and airway leakiness.	25957295 [156]
Asthma	NRF2-KO vs NRF2-WT	Exposition to chlorine gas through the nose for 5mins. SFN (10 mg/kg) injected i.p once/day for 4 days before exposure. Measurements performed 24h or 48h post-exposure.	NRF2-KO mice showed a more prolonged airway hyperresponsiveness and inflammation in response to Chlorine gas exposure. SFN increases glutathione levels and reduces airway hyperresponsiveness and inflammation in wild type, but not in NRF2-KO, mice.	27847240 [469]
	Wild type	Mice sensitized on day 0 and 14 by subcutaneous administration of OVA (100 μg) and challenged by intranasal administration of OVA (10 μg) on day 28. Some OVA-challenged mice were exposed to whole body exposure to smoke from 10 cigarettes for 4 consecutive days on day 26. Mice were treated i.p with 25 mg/kg of the corticosteroid dexamethasone on day 29 and with SFN (12.5 mg/kg) on days 26–29	SFN increased antioxidant gene expression and reversed the corticosteroid insensitivity induced by CS on OVA-exposed mice.	30312763 [470]
	Wild type	Mice sensitized by intranasal administration of CE (Blattella germanica) allergen extract (50 μg/25 μl) on days 1, 2, 3, 4, and 5. Mice were challenged with CE (25μg/25 μl) twice/day on days 11–17. SFN (25 mg/kg) was administered i.p once/daily before CE challenge. Dexamethasone (1 mg/kg) was administered intranasally once/day 1h before CE challenge.	Sulforaphane increased antioxidant enzyme expression and reduced Th17-driven inflammation in CE-exposed mouse lungs. Sulforaphane also potentiated the anti-inflammatory effects of the corticosteroid dexamethasone.	31100413 [471]
	Wild type	Mice were sensitized with OVA (25 μg/200 μl) on days 0, 7 and 14. Following the initial sensitization mice were challenged with aerosolized OVA (2 %) for 30 min once/day on days 21–28 and 49. 1h following the last exposure mice were exposed to ozone gas mixed with air (3 ppm) for 3h in a sealed container. Some mice were treated i.p with a single dose of RTA-408 (17.5 mg/Kg) 24h prior to ozone exposure.	RTA-408 reduced OVA/Ozone-induced oxidative stress and airway inflammation, hyperresponsiveness and remodeling.	31256292 [163]
COPD	NRF2-KO vs NRF2-WT	Exposition to CS (5 cigarettes at one time; 2.45 mg nicotine per cigarette) in a chamber for 7 h/day, 7 days/week, for up to 6 months	NRF2-KO mice showed early onset and more extensive emphysema, bronchoalveolar oxidative stress and inflammation, and increased alveolar epithelial cell apoptosis in response to CS. NRF2-KO mice also had attenuated antioxidant gene expression.	15520857 [170]

(*continued on next page*)

Table 4 (*continued*)

Study object	Model	Treatment	Results	PMID
	NRF2-KO vs NRF2-WT	Intratracheal instillation of 25 μg of porcine pancreatic elastase	NRF2-KO mice showed a greater acute inflammatory response associated with more pronounced elastase-induced emphysema. NRF2-KO mice also demonstrated lower antioxidant and anti-protease gene expression.	16272357 [176]
	NRF2-KO vs NRF2-KO	Exposition to CS (5 cigarettes at one time; 19 ng/puff, nicotine 1.3 mg/puff) in a chamber for 50min/day, 4 days/week, for 16 weeks	NRF2-KO mice showed emphysema 8 weeks post-exposure, whilst WT mice did not show emphysema. NRF2-KO show augmented neutrophilic lung inflammation, reduced antioxidant gene expression and increased oxidative stress.	16324149 [34]
	NRF2-KO vs NRF2-WT	Exposition to CS (tar 9.7 mg/cigarette, nicotine 0.85 mg/cigarette) in a chamber for 1h/day, with 1h interval in between for 3 days. Alternatively, mice were exposed to o aerosolized LPS (1 mg/ml) for 8 min. In some mice, the corticosteroid budesonide (1 or 3 mg/kg) was administered intranasally for 3 days followed by LPS exposure.	NRF2-KO mice were more susceptible to CS-induced inflammation than NRF2-WT. NRF2-KO mice were less responsive to the anti-inflammatory effects of budesonide following LPS exposure than NRF2-WT.	21094147 [472]
COPD	NRF2-KO vs NRF2-WT	Exposition to CS in a chamber for 2.5 h/day, 5 days/week, for 1 week or 6 months. Some mice were exposed to nebulized SFN (0.5mg/fay) following CS exposure.	Alveolar macrophages from CS-exposed mice showed reduced bacterial phagocytosis at 1 week and 6 months post-exposure. This effect was augmented in NRF2-KO mice. SFN improved phagocytosis in WT mice but not in NRF2-KO mice.	21490276 [172]
	NRF2-KO vs NRF2-WT	Exposition to CS (10 cigarettes/day for 4 consecutive days) and intranasally inoculated with Influenza virus (250 PFU) after 4 days.	CS-exposed NRF2-KO mice showed attenuated antioxidant gene induction, and higher parabronchial inflammation, lung damage and mucus hypersecretion and mortality rates after influenza virus infection. Lung oxidative stress and inflammatory gene expression was increased in NRF2-KO mice.	21367886 [171]
	NRF2-KO vs	Exposition to CS (Kentucky	CS-exposed NRF2-KO mice showed greater	23559007

Table 4 (*continued*)

Study object	Model	Treatment	Results	PMID
	NRF2-WT	reference cigarette 3R4F) for 5h/day for 4 days.	oxidative stress-induced lung inflammation, alveolar type II epithelial cell damage and apoptosis.	
	NRF2-KO vs NRF2-WT	Exposition to CS (Kentucky reference cigarette 3R4F) for 5h/day for 3 days. Another group was treated with 0.5 g/Kg NAC every 24h, starting 1 day before CS exposure and for 3 days during CS exposure.	CS-exposed NRF2-KO mice showed greater lung oxidative stress, inflammation and alveolar type II epithelial cells. NAC protected WT, but not NRF2-KO mice, from CS-induced alveolar epithelial cell apoptosis.	23492188 [473]
	NRF2-KO vs NRF2-WT	Wild type and NRF2-KO mice were intratracheally injected with porcine pancreatic elastase (80U/Kg) once. Some animals received LiCl (200 mg/Kg/day) for 7 days via intraperitoneal injection. Analysis was performed 21 days-post elastase exposure.	LiCl decreased elastase-induce emphysema and lung inflammation in WT, but not NRF2-KO mice. LiCl activated Wnt signalling and NRF2 expression in WT, but not NRF2-KO mice.	29659176 [474]
	NRF2-KO vs NRF2-WT	Intratracheal instillation of particulate matter (PM; 200 μg). Some mice were treated with the H2S donor NaHS (50 μM/Kg) or the H2S blocker propargylglycine by intraperitoneal injection 30min before PM administration. Animal were sacrificed 29 days before administration.	NaHS inhibited PM-induced emphysema, airway inflammation in WT but not in NRF2-KO mice. NaHS also prevented reactive oxygen species production, NLRP3 activation and apoptosis in the lungs of WT, but not of NRF2-KO, mice.	32116706 [177]
COPD	NRF2-KO vs NRF2-WT	Exposition to CS (15 cigarettes, 1h, 6 times/day) in a chamber for 90 days. 24h post-exposure, some mice were treated with allyl isothiocyanate (20 mg/Kg) or N-acetyl cysteine (800 mg/Kg) twice/day for 15 days.	CS-exposed NRF2-KO mice showed greater emphysema and lung inflammation. Allyl isothiocyanate prevented the effects of CS in WT, but not in NRF2-KO, mice. Bronchial epithelial cells from NRF2-KO mice showed reduced expression of Notch1, Hes1 and multidrug resistance-associated protein 1 (MRP1). In vitro experiments showed that allyl isothiocyanate confers its antioxidant and anti-inflammatory effects by inducing MRP1 in an NRF2/Notch1-dependent mechanism.	31927049 [475]

(*continued on next page*)

Table 4 (*continued*)

Study object	Model	Treatment	Results	PMID
	NRF2-KO vs NRF2-WT	Exposition to ambient PM2.5 using specially designed chambers 24h/day, 7 days/week for a total of 6 weeks.	PM-induced lung injury, cell apoptosis and inflammation were attenuated in the lungs of NRF2-KO mice. PM exposure increased oxidative stress levels in the lungs of both WT and NRF2-KO mice. PM-exposed NRF2-KO mice showed lower expression of the detoxification enzyme CYP2E1 and reduced markers of ER stress.	33967806 [178]
	NRF2-KO vs NRF2-WT	Exposition to PMv2.5 (200 μg) by intratracheal instillation for 29 days. Some mice were treated, 30 min before PM exposure, with the H2S donor NaHS (50 μM/Kg) by intraperitoneal injection.	PM-induced emphysema, lung inflammation and expression of lipid peroxidation and ferroptosis markers was more pronounced in NRF2-KO mice. NaHS protected against PM-induced emphysema, inflammation and ferroptosis in WT but not in NRF2-KO mice. In vitro experiments showed that the inhibitory effect of NaHS is mediated by a NRF2/PPAR-γ pathway.	35490984 [179]
	Wild type	Exposition to CS [5 cigarettes (with a 5 min smoke-free break between exposure to each cigarette); 1 mg nicotine, 11 mg CO, 10 mg tar) in a chamber 5 days/week for a total of 16 weeks. DHS was administered before CS exposure by intraperitoneal injection (2 or 4 mg/Kg), 4 days/week for a total of 16 weeks.	DHS treatment prevented CS-induced emphysema, fibrosis and inflammation in the mouse lungs. DHS also increased NRF2 and antioxidant protein levels and reduced oxidative stress markers and the activation of the pro-inflammatory transcription NFκB in the lungs of CS-exposed mice.	31760092 [175]

responses, lung injury and mortality in response to influenza virus infection [171]. Alveolar macrophages from NRF2-KO mice were also shown to have reduced ability to phagocytose bacteria, due to reduced expression of the scavenger receptor MARCO [172]. These findings indicate a protective effect of NRF2 against epithelial damage and abnormal immune activation, slowing down the development of emphysema. Furthermore, NRF2 may also regulate immune responses against infections, therefore, reducing the susceptibility of patients to disease exacerbations. N-acetyl cysteine was shown to prevent airway epithelial damage in NRF2-KO mice, suggesting that the protective effect of NRF2 may be, at least partly, mediated by its antioxidant effects [173]. The natural compounds SFN, allyl isothiocyanate and *trans*-4, 4′-dihydroxystilbene were also shown to reduce lung inflammation and emphysema, and normalize immune responses, in CS-exposed mice in an NRF2-dependent manner [172,174,175]. A similar protective effect of NRF2 was also shown in the elastase-induced model using NRF2-KO mice [176].

NRF2 was also shown to have an anti-inflammatory effect and to protect against epithelial injury and the development of emphysema in models of intratracheal instillation or whole-body exposure to particulate matter [177–179]. In the same model, hydrogen sulfide was shown to prevent NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation, lung epithelial cell ferroptosis, inflammation, and emphysema, at least partly, through NRF2-mediated antioxidant protection [177].

The above studies, summarized in Table 4, demonstrate the potential of using mouse models for investigating the role of NRF2 in COPD pathogenesis, and for validating new therapeutic targets. Whilst the role of NRF2 in the protection against lung epithelial oxidative damage and the development of emphysema has been established, a better understanding of its role in other aspects of COPD pathology such as airway remodeling, disease exacerbations, as well as in early life exposures, is required. Finally, the mechanisms underlying the effects of NRF2 on COPD pathogenesis need to be explored further, beyond just its antioxidant effects. Mitochondrial dysfunction and metabolic reprogramming may play a key role in COPD pathogenesis, suggesting that the effects of NRF2 on mitochondrial biogenesis and cell metabolism may be crucial [180–183].

4.4.3. *Mouse models of cardiovascular pathologies*

An imbalance between ROS production and the antioxidant defense system is central to the pathogenesis of cardiovascular disease. Genetically modified mice lacking NRF2 have shed light on the role of this factor in cardiovascular diseases, including cardiac hypertension, heart failure and ischemia-reperfusion (IR) injury (Table 5). NRF2-KO mice have a left ventricular (LV) diastolic dysfunction, characterized by prolonged E wave deceleration time, relaxation time and total diastolic time, increased E/A ratio and myocardial performance index. LV dysfunction in NRF2-KO mice was associated with cardiac hypertrophy and a downregulation of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) in the myocardium. However, vascular function was fully preserved, blood pressure was decreased, and endothelial nitric oxide synthase (eNOS) was upregulated in the aorta and in the heart of NRF2-KO mice [184]. Hearts of NRF2-KO mice had impaired antioxidant gene expression, increased hypertrophy, and impaired function compared with those of wild-type littermates following chronic pressure overload. The authors identified the ROS-generating enzyme nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4)-dependent upregulation of NRF2 as an endogenous protective pathway that limits mitochondrial damage and apoptosis-inducing factor (AIF)-related cell death in the heart under hemodynamic overload. In mice with cardiac-specific overexpression of NOX4, NRF2 deletion significantly attenuated their protective phenotype during chronic pressure overload [185]. Cui and colleagues studied the effect of cardiomyocyte-specific transgenic activation of NRF2 on cardiac pathological remodeling and dysfunction [186]. Cardiomyocyte-specific activation of NRF2 ($NRF2^{ctg}$ mice) suppressed myocardial oxidative stress, cardiac apoptosis, fibrosis, hypertrophy and dysfunction under sustained pressure overload induced by transverse aortic arch constriction (TAC). The constitutive activation of NRF2 increased the steady-state level of autophagosomes while decreasing the ubiquitinated protein aggregates in the heart after TAC. Therefore, NRF2 attenuated the proteocytotoxicity presumably via enhancing autophagy-mediated clearance of ubiquitinated protein aggregates in cardiomyocytes. The same authors reported later both protective and detrimental effects of NRF2 in the heart depending on the functional integrity of autophagy. Thus, in a murine model of pressure overload-induced cardiac remodeling and dysfunction via TAC, the absence of NRF2 enhanced myocardial necrosis and death rate during an initial stage of cardiac adaptation when myocardial autophagy is intact. However, the lack of NRF2 became cardioprotective in the later stage of cardiac remodeling when autophagy is insufficient. The authors concluded that NRF2 is required for cardiac adaptive responses when

Table 5
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in cardiovascular diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are either null expression models for NRF2 (pink), models employing NRF2 overexpression (green), and those in which NRF2 has been activated employing pharmacological strategies (blue) or indirect methods (grey). *Dimethyl fumarate (DMF); ischemia/reperfusion (IR); intraperitoneal injection (i.p); intravenous injection (i.v.); left anterior descending artery (LAD); lipopolysaccharides (LPS); transverse aortic constriction (TAC); oral administration (p.o.); subcutaneous injection (s.c.).*

Study Object	Model	Treatment	Results	PMID
Cardiovascular diseases	NRF2-KO vs NRF2-WT	None	Left ventricular diastolic dysfunction. Cardiac hypertrophy and downregulation of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a). Fully preserved vascular function, decreased blood pressure and a compensatory increase in eNOS expression in the aorta and in the heart.	26475037 (184)
	NRF-KO vs NRF2-WT	Chronic pressure overload	Greater cardiac dilation and contractile impairment than wild types. NRF2-KO mice developed greater cardiac hypertrophy than WT. NRF2 deficiency increased myocardial oxidative stress, mtDNA damage and caspase 3-independent cell death.	25534702 (185)
	NRF2-KO vs NRF2-WT	TAC-pressure overload	The absence of NRF2 enhanced myocardial necrosis and death rate when autophagy was intact but became cardioprotective when autophagy was insufficient.	26573705 (187)
	NRF2-KO vs NRF2-WT	IR injury, LAD coronary artery occlusion (30′I/24 h R) ischemic preconditioning (2 × 5′I/5′R)"	Mice lacking NRF2 are more sensitive to IR injury and present reduced protection by ischemic preconditioning.	24915518 (190)
	NRF2-KO vs NRF2-WT	4-HNE treatment (4 mg/kg i.v. Body weight); ex vivo IR (25′I/60′R)	4-HNE activated NRF2 in the heart, increased myocardial GSH content, and improved the functional recovery of the LV following IR in Langendorff perfused wild-type hearts. The cardioprotective effect of 4-HNE was lost in NRF2-KO mice.	20685357 (194)
	NRF2-KOvs NRF2-WT	TAC-induced cardiac hypertrophy	NRF2-KO mice developed cardiac hypertrophy, myocardial fibrosis and apoptosis, heart failure and increased mortality after TAC.	19592468 (476)
	NRF2-KO vs NRF2-WT	Heterotopic heart transplantation	NRF2-KO showed significantly less SOD1/2 activity, high mRNA levels of NK-kB-mediated proinflammatory genes graft dysfunction, apoptosis and cardiac allograft vasculopathy.	31769924 (197)
	NRF2-KO vs NRF2-WT	In vivo IR (30′I/24 h R)	NRF2-KO mice had a smaller infarct size following myocardial IR than wild-type mice and showed fully preserved LV systolic function due to eNOS upregulation.	29854098 (199)
Cardiovascular diseases	NRF2-KO vs NRF2-WT	DMF (15 mg/kg, p.o. gavage twice daily for 5 days) followed by left coronary artery ligation for 24 h	DMF stabilized and increased NRF2 protein in wild-type mice. DMF significantly reduced the infarct size in wild-type but not in NRF2-KO mice.	22405071 (204)
	$NRF2^{ctg}$ vs NRF2-WT	TAC-pressure overload	Suppression of myocardial oxidative stress, cardiac apoptosis, fibrosis, hypertrophy and dysfunction. Attenuation of proteotoxicity presumable via enhancing autophagy-mediated clearance of ubiquitinated protein aggregates.	24747945 (186)
	mNRF2-^{TG} vs NRF2-WT	ISO-induced oxidative stress (50 mg/kg body weight s.c. once daily for 7 days)	Preservation of myocardial structure and function associated with the enhanced NRF2 expression and with an increased expression of redox modulatory genes and an overall enhanced antioxidant status.	31155513 (188)
	Wild type	19a (Keap1-NRF2 PPI inhibitor; 10 mg/kg) followed by LPS-induced injury (15 mg/kg LPS, i.p.)	19a increased the protein levels of NRF2 together with its downstream cytoprotective proteins. 19a protects against LPS-induced heart tissue injury and inflammation *in vivo*. 19a reduced ROS levels elevated by LPS and increased the GSH/GSSG ratio.	32902980 (208)
	Wild type	Ischemic preconditioning (2 × 5′I/5′R)	Rapid elevation of NRF2 protein because of de novo protein translation in the myocardium.	24915518 (190)
	Wild type	IR (45′I/2 h R) and intracoronary delivery of AAV- shHDAC9 or AAV-shCTRL	Knockdown of HDAC9 activated the KEAP1-NRF2-HO-1 pathway, which further promoted the proliferation and myogenesis and inhibited the apoptosis of cardiomyocytes.	33963859 (198)
	Nox4^{TG}	Chronic pressure overload	The stress-induced upregulation of NRF2 observed in wild-types after aortic constriction was completely abolished in Nox4-KO mice.	25534702 (185)

autophagy is intact; however, NRF2 mediates cardiac maladaptive remodeling and dysfunction when autophagy is impaired [187].

Cardiac-specific NRF2 transgenic mice (mNRF2-TG) were resistant to isoproterenol (ISO)-induced oxidative stress, suppressing pathological remodeling [188]. Preservation of myocardial structure and function in the mNRF2-TG mice was associated with the enhanced NRF2 expression displayed in these hearts with an increased basal and post-treatment expression of redox modulatory genes and an overall enhanced antioxidant status. The role of NRF2 in cardiac IR injury has been recently reviewed [189]. Myocardial reperfusion after ischemia induces de novo synthesis of NRF2, which protects against IR injury [190]. Similarly, reperfusion-specific activation of NRF2 has been reported in renal epithelial cells exposed to hypoxia/reoxygenation [191]. In the same study, the antioxidant N-acetyl cysteine (NAC), which attenuates ROS overproduction in reoxygenation, suppressed NRF2 activation, suggesting that ROS mediate NRF2 signaling. Similarly, activation of the NRF2 pathway by miRNAs protects murine cardiomyocytes against in vitro IR or oxygen and glucose deprivation (OGD) followed by reperfusion [192,193]. Using a model of left anterior descending (LAD) coronary artery occlusion, Xu et al. showed that NRF2-KO mice present with increased infarct area relative to wild type mice, supporting a protective role for NRF2 against cardiac IR injury [193]. NRF2-KO mice also showed a reduced degree of cardiac protection by means on ischemic preconditioning [190]. In addition, the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE) induced NRF2 activation in mouse cardiomyocytes [194,195], which protected them against glucose-free anoxia followed by reoxygenation. Likewise, intravenous administration of 4-HNE activated NRF2 in the heart, increasing glutathione content, and improved left ventricular functional recovery following IR in Langendorff-perfused hearts from wild type but not from NRF2-KO mice [193]. Activation of NRF2 has also been reported to protect the murine heart against pathological cardiac hypertrophy and heart failure by suppressing oxidative stress after TAC [196]. In murine heterotopic heart transplant models, NRF2 was shown to

inhibit NF-κB activation and protect against IR injury, which was mediated by its antioxidant activity, and suppress the subsequent development of cardiac allograft vasculopathy [197]. Similarly, histone deacetylase 9 (HDAC9) knockdown induced the activation of NRF2 and protected the heart from myocardial infarction. In addition, HDAC9/NRF2 axis modulated the proliferation, apoptosis and myogenesis of mouse neonatal cardiomyocytes [198]. Contrary to most studies, which show a protective effect of NRF2 activation against IR injury, Erkens et al. reported that global NRF2 gene knockout attenuates IR myocardial injury and dysfunction in mice, most likely due to cardioprotection by nitric oxide derived from endothelial nitric oxide synthase (eNOS) [199]. Numerous studies have focused on the potential beneficial effects of NRF2-mediated activation of endogenous antioxidant defenses. Thus, many phytochemicals and related compounds such as SFN and carnosic acid, activate NRF2 to increase its cytoprotective programme that play significant roles not only in cardioprotection but also in the prevention of IR injury in other organs [200–203]. The electrophilic inhibitor of KEAP1 dimethyl fumarate (DMF), an approved therapeutic for multiple sclerosis, has shown protective effects against cardiac ischemia *in vivo* [204] and in vitro [205]. Electrophiles, however, may react with several nucleophiles within the cell. A class of NRF2 inducers (non-electrophilic modulators) that prevent the interaction between NRF2 and KEAP1, known as protein-protein interaction (PPI) inhibitors for KEAP1-NRF2 are a focus of intense research for their therapeutic potential [206,207]. Such is the case of 19a, an indoline-based KEAP1-NRF2 PPI inhibitor, that has shown protective effects in both rat H9c2 cardiac cells and an *in vivo* mouse model of LPS-induced cardiac injury [208]. Taken together, NRF2 activation plays a protective role in heart disease, although it can be detrimental under specific conditions.

4.4.4. Mouse models of type 2 diabetes mellitus

The most common metabolic disease is diabetes mellitus type 2 (T2D), which is defined as a chronic metabolic disorder characterized by defects in insulin secretion and/or action, thus resulting in chronic hyperglycemia [209]. Prolonged hyperglycemia, excessive lipid content, mitochondrial dysfunction and enhanced formation of advanced glycation end products (AGEs) lead to ROS production [210] which actively contribute to T2D progression [211].

Recent advances in basic and translational research have provided insights into the critical role of the KEAP1-NRF2 pathway in T2D and its comorbidities [212–214]. In the last decades, several mouse models have been widely used to decipher the impact of modulating NRF2 levels in the context of insulin resistance and diabetes (Table 6).

The impact of genetic activation of NRF2 on T2D was evaluated using KEAP1 knockdown mice crossed with the commonly known db/db mice. These animals express a truncated version of the leptin receptor becoming especially susceptible to obesity, insulin resistance and T2D. Interestingly, db/db:KEAP1$^{flox/-}$ mice did not show hyperglycemia and glucose intolerance when compared to their corresponding controls (db/db:KEAP1$^{flox/+}$ mice) and, importantly, an improvement in insulin secretion after glucose stimulation was observed in db/db:KEAP1$^{flox/-}$ mice, strongly suggesting that NRF2 is likely involved in glucose metabolism [215]. At the molecular level, NRF2 upregulation has been reported to protect pancreatic islets by activating expression of antioxidant genes. These results were further confirmed by inducing NRF2 signaling with CDDO-Im. NRF2 pharmacological activation strongly ameliorated T2D-induced metabolic effects through both beta cell protection against oxidative stresses and improvement of insulin resistance in the liver and skeletal muscle. Nevertheless, contradictory results emerged from an earlier study from Slitt's group [216]. Contrarily to the db/db:KEAP1$^{flox/-}$ mice, KEAP1 knockdown in an ob/ob background (Lep$^{ob/ob}$-KEAP1-KD mice) impaired insulin signaling, prolonged hyperglycemia, and dampened insulin resistance which was accompanied by reduced peripheral glucose and lipid metabolism [216]. It is important to highlight that whereas both ob/ob and db/db mice develop comparable obesity, db/db mice are more diabetic than ob/ob mice [217]. From the discrepancies between the abovementioned studies arise the possibility that KEAP1-NRF2 modulation might be differentially controlled during diabetes onset and

Table 6
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in Type 2 diabetes mellitus. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null expression models for NRF2 (pink) and models employing NRF2 activation by knocking down Keap1 expression (green). *High fat diet (HFD).*

Study object	Model	Treatment	Results	PMID
Type 2 diabetes mellitus	NRF2-KO vs NRF2-WT	HFD administration for 12 weeks	Mice resistant to HFD-induced glucose intolerance	23017736 [221]
	db/db:NRF2-KO vs db/db:NRF2-WT	None	Reduced adipogenesis. Impaired insulin signalling	25270507 [222]
	ob/ob:NRF2-KO vs ob/ob:NRF2-WT	None	Impaired glucose tolerance and aggravated hyperglycemia	23238296 [226]
	Nrf2$^{flox/flox}$:: MIP-CreER^{TAM} (Beta cell-specific NRF2-conditional knockout mice)	None	Reduced beta-cell mass. Decreased insulin content in the islets after a HFD	35192689 [219]
	ob/ob::Nrf2-KO::Fabp4/aP2-Cre (Adipose tissue-specific NRF2 deficiency in the ob/ob background)	None	Impaired glucose tolerance and aggravated hyperglycemia	23238296 [226]
	Keap1$^{flox/flox}$ (Keap1-hypo) (hypomorphic KEAP1 alleles)	None	Suppressed hepatic gluconeogenesis and lipogenesis after a HFD administration for 12 weeks	26701603 [220]
	db/db::Keap1$^{flox/-}$ and db/db::Keap1$^{flox/+}$ control mice	None	Absence of hyperglycemia and glucose intolerance. Improved insulin secretion. Increased hepatic FGF21 levels	23716596 [215], 25270507 [477]
	Lep$^{ob/ob}$-Keap1-KO	None	Impaired insulin signaling, prolonged hyperglycemia and worsened insulin resistance	22936178 [216]
	RIP-Cre::Keap1$^{flox/flox}$::Nos2-Tg (Beta cell-specific KEAP1 knockdown in iNOS-Tg background)	None	Preservation of the insulin-positive area of the pancreas and protection against apoptosis	24186865 [218]
Type 2 diabetes mellitus	*Keap1$^{flox/flox}$:: MIP-CreER^{TAM}* (Beta cell-specific KEAP1-conditional knockout mice)	None	Increased beta-cell mass. Improved glucose tolerance upon a HFD administration	35192689 [219]
	Keap1$^{flox/flox}$::Alb-Cre mice (Liver specific KEAP1 knockdown mice)	None	Blood glucose levels were slightly decreased	23716596 [215]
	Keap1$^{flox/flox}$::MuCreA mice (Skeletal muscle specific KEAP1 knockdown mice)	None	Reduced blood glucose levels and improved insulin sensitivity	23716596 [215], 27044864 [227]
	Keap1-Trsp^{RIP}KO mice (KEAP1 deficiency in hypothalamus and pancreas)	None	Improved insulin sensitivity	28228267 [228]

progression. At present, this issue remains unclear and needs to be clarified in future studies.

A follow-up study by Yagishita et al. [218] used several beta cell-specific conditional KO mice to deplete either KEAP1 or NRF2 in this cell type. In this work, they found that genetic NRF2 induction by KEAP1 depletion strongly preserves the insulin-positive area of the pancreas and protects against apoptosis in an iNOS (*Nos2*)-Tg background, a beta cell-specific reactive species-mediated damage model. On the contrary, beta cell-specific NRF2-conditional KO mice exhibited reduced islet size and impaired glucose tolerance in response to oxidative stress. Supporting these results, a more recent study described that NRF2 overexpression induced by KEAP1 deficiency protects beta cells from apoptosis and increases beta cell mass and proliferation ratio, as well as improves glucose tolerance upon a high fat diet (HFD) administration [219]. Conversely, mice lacking NRF2 in beta cells showed decreased adaptive beta cell proliferation and mass expansion in addition to reduced insulin content in the pancreatic islets after a HFD feeding. Notably, NRF2 deletion also contributed to diminish the mRNA expression of some beta cell identity genes, such as *Pdx1*, *Ins2*, *Glut2*, and *Nkx6.1*, because of oxidative stress and ROS generation.

Besides the pancreas, the potential role of NRF2 in mitigating insulin resistance and its detrimental effects in insulin-target tissues has been getting more attention in the last years. Slocum et *al.* [220] demonstrated that NRF2 induction by using the KEAP1-hypo ($KEAP1^{flox/flox}$) mouse model, in which KEAP1 alleles are hypomorphic, suppressed hepatic gluconeogenesis as well as lipogenesis in after a HFD administration, thus ameliorating the diabetic phenotype of the KEAP1-hypo mice induced by this obesogenic diet. Of note, these results contradict those previously reported for the NRF2-deficient mice which were resistant to HFD-induced glucose intolerance [221]. A plausible explanation for these divergences between both studies could be attributed to the diet composition since they differed in the percentage of kcal derived from fat (60 % *versus* 40 %, respectively) and in the sources of fat (lard *versus* hydrogenated vegetable oil, butter fat, corn oil, and soy oil, respectively).

Another study in db/db:$KEAP1^{flox/-}$ mice [222] described an increase in Fibroblast growth factor 21 (FGF21) in their livers but not in other tissues such as adipose tissue and pancreas, thereby identifying NRF2 as a positive regulator of this hepatokine. FGF21 has been pointed as a potential target for metabolic disorders since higher circulating levels improves hyperglycemia, insulin resistance and hyperlipidemia and attenuates weight gain in diabetic mouse models [223,224]. In agreement with these data, CDDO-Im administration increased both hepatic *Fgf21* expression and plasma FGF21 levels in db/db mice but not in NRF2-deficient db/db mice, evidencing the importance of NRF2 in FGF21 regulation in a diabetic context. However, controversial results have been reported regarding the impact of NRF2 in FGF21 signaling pathway. In a HFD-induced obesity model, both *Fgf21* mRNA and protein were elevated in livers of NRF2-deficient mice, whereas FGF21 protein levels were lower in KEAP1-knockdown mice than in wild-type mice [221], showing an inverse correlation between NRF2 levels and hepatic FGF21 that might explain the improved glucose tolerance in HFD-fed NRF2-null mice. It is important to mention that distinct alterations in metabolic profile were evident when compared genetic (db/db mice) and diet-induced obesity mice models. For instance, circulating insulin levels are elevated in both models of obesity, while glucagon was increased only in the db/db mice [225], suggesting that the db/db mice are more closely related to human T2D whereas the diet-induced obesity models represent the human prediabetic condition.

NRF2 activation in the db/db:$KEAP1^{flox/-}$ mice also elicited an enhanced expression of glucose and lipid metabolism-related genes in adipose tissue, which in turn improved plasma lipid profiles in these mice [222]. In line with these evidence, ablation of NRF2 globally or specifically in adipocytes on the leptin-deficient ob/ob background resulted in aggravated insulin resistance, hyperglycemia, and hypertriglyceridemia [226].

Skeletal muscle is essential for glucose metabolism, being responsible for 80 % of postprandial glucose uptake from the circulation. As insulin resistance in the skeletal muscle appears prior to beta cell failure, the impact of modulating KEAP1-NRF2 specifically in this tissue has also been studied by using skeletal muscle-specific KEAP1-KO mice [215,227]. Notably, NRF2 activation in skeletal muscle reduced blood glucose levels both under feeding conditions and during an insulin tolerance test (ITT) by increasing glucose uptake and suppressed body weight gain in upon a HFD administration. Since liver-specific KEAP1 knockdown mice have been reported to show only a slight decrease in blood glucose levels under feeding conditions, it is tempting to assume that NRF2 activation in skeletal muscle mainly contributes to reduce blood glucose levels after an insulin stimulation, thus being able to hypothesize that the improved insulin sensitivity observed in the ITTs of the global KEAP1 knockdown mice are mainly due to the activation of NRF2 in the muscle [215].

Finally, a recent study in a murine model that depletes KEAP1 in both hypothalamus and pancreas [228] revealed that increased levels of hypothalamic NRF2 improved peripheral insulin sensitivity by suppressing oxidative stress in this brain region, thus suggesting that central activation of NRF2 reduced hypothalamic oxidative damage thereby preventing the onset of T2D.

Overall, all these preclinical studies in mice point NRF2 as a key player in the onset and progression of T2D and make the KEAP1-NRF2 pathway a promising candidate for the development of future therapeutics for the treatment of T2D. However, given the complexity of this disease, there are still a number of discrepancies that require further investigation.

4.4.5. Mouse models of liver disease

Metabolic dysfunction-associated steatotic liver disease (MASLD; formerly known as non-alcoholic fatty liver disease, MAFLD) is the most common liver disorder, affecting about 25 % of the global population [229]. MASLD is marked by excessive fat accumulation in the liver without a history of alcohol use or other liver diseases When liver fat exceeds 5 % and is accompanied by inflammation and swelling of hepatocytes, it progresses to metabolic dysfunction-associated steatohepatitis (MASH; formerly known as nonalcoholic steatohepatitis, NASH). MASH causes the progressive death of hepatocytes, leading to fibrosis, cirrhosis, and even hepatocellular carcinoma [230,231].

The mechanisms underlying the development and progression of MASH are complex and multifactorial. These include hepatic steatosis, primarily characterized by an excess of triacylglycerides, hepatocellular injury, mitochondrial dysfunction, excessive production of ROS beyond homeostatic capacity, and the presence of inflammatory infiltrates and pro-inflammatory signaling. MASH results in liver fibrosis, which is the primary cause of mortality in MASH patients [232]. Multiple preclinical models of MASH have been proposed and can broadly be induced by diet, toxins, genetically, or a combination of these. The major limitation of these models is the development of advanced fibrosis, which is problematic since fibrosis stage is considered the best prognostic marker in patients and an important endpoint in clinical trials of MASH. Proof-of-concept studies suggest that NRF2 activation may combat several underlying mechanisms of MASH in one approach (Table 7).

Mice fed HFD (up to 70 % of calories from fat) developed steatosis within 10 weeks, mild inflammation after 34 weeks, and slight perivenular fibrosis after 50 weeks [233]. In these mice, expression of NRF2 and its target genes were reduced and associated with elevated serum and hepatic levels of cholesterol and triglycerides. In contrast, mice deficient for NRF2 had higher levels of lipids and lipid peroxidation in the liver than wild type [234]. Proteomic analysis identified cellular defense and lipid metabolism as key NRF2-related pathways in the liver. Loss of NRF2 resulted in a down-regulation of proteins involved in cellular defense such as glutathione transferases and glucuronic transferases and in a large up-regulation of proteins involved in the regulation of lipid, protein and carbohydrate metabolism. For instance, the

Table 7

Examples of studies using *M musculus* as model organism to examine the role of NRF2 in liver diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null expression models for NRF2 (pink), models employing NRF2 overexpression (green), and those in which NRF2 has been stimulated employing classical activators (blue) or natural derived products (grey). *Advanced glycation end products (AGEs); Amylin liver NASH (AMLN); Carbon monoxide releasing molecule-A1 (CORM); high fat diet (HFD); High fat plus fructose (HF/HF); metabolic dysfunction-associated steatohepatitis (MASH); Metabolic associated fatty liver disease (MAFLD); Methionine/Choline Deficient diet (MCD diet); intraperitoneal injection (i.p.); Citrus reticulata Blanco (Jizigan) peel extract (JZE); oral administration (p.o.).*

Study object	Model	Treatment	Results	PMID
MASH/ MAFLD	NRF2-KO vs NRF2-WT	None	NRF2-KO mice exhibited a profound alteration on the expression levels of genes involved in the synthesis and metabolism of fatty acids and other lipids including the gene coding for ATP-citrate lyase, responsible for acetyl-CoA production.	20399915 (235)
	NRF2-KO vs NRF2-WT	HFD for 4 weeks	HFD reduced the mRNA expression of NRF2 and its target genes. The mRNA levels of lipogenic and cholesterologenic transcriptional factors and their target genes were higher in NRF2-null mice compared with wild-type mice after feeding an HFD. Hepatic free fatty acid and malondialdehyde equivalents were higher in NRF2-null mice compared with wild-type mice.	18281592 (234)
	NRF2-KO vs NRF2-WT	HFD for 27 weeks	NRF2-KO mice were partially protected from HFD-induced obesity and developed a less insulin-resistant phenotype. Importantly, NRF2-KO mice had higher plasma FGF21 levels and higher FGF21 mRNA levels in liver and white adipose tissue than WT mice.	21852674 (242)
	NRF2-KO vs NRF2-WT	HFD for 24 weeks	NRF2-KO mice displayed more severe MASH, with cirrhosis. NRF2 protects against MASH by suppressing lipogenesis, supporting mitochondrial function, increasing the threshold for the UPR and inflammation, and enabling adaptation to HFD-induced oxidative stress	24958099 (240)
	NRF2(L)-KO and NRF2(Mϕ)-KO (Hepatocytes and macrophage specific NRF2-KO)	HFD for 12 weeks	NRF2(L)-KO mice showed less liver enlargement, milder inflammation and less hepatic steatosis after HFD feeding. In contrast, NRF2(Mϕ)-KO mice displayed no significant difference in HFD-induced hepatic steatosis from Nrf2-LoxP control mice.	31901728 (241)
	NRF2-KO vs NRF2-WT	MCD diet for 2 weeks	Livers from NRF2-KO mice showed a substantial increase in steatosis and a massive increase in the number of neutrophil polymorphs, compared to livers from wild-type mice treated similarly. Livers of NRF2-KO mice suffered more oxidative stress than their wild-type counterparts and heightened inflammation.	19914374 (244)
	NRF2-KO and p62-KO vs NRF2-WT	Arsenic in the drinking water for 20 weeks	Prolonged non-canonical NRF2 activation via p62-mediated sequestration of KEAP1 increases carbohydrate flux through the polyol pathway, resulting in a pro-diabetic shift in glucose homeostasis, through the upregulation of four novel NRF2 target genes *Khk, Sord, Tkfc* and *Hnf4A*.	33933676 (243)
	NRF2-KO vs NRF2-WT	Cholesterol diet for 4 weeks	Dysregulated lipid and bile acid metabolism and hepatic steatosis	24688217 (237)
	NRF2-KO vs NRF2-WT	HFD or HFD+0.3%glucoraphanin for 14 weeks	Glucoraphanin supplementation attenuated weight gain, decreased hepatic steatosis, and improved glucose tolerance and insulin sensitivity in HFD-fed wild-type mice but not in HFD-fed NRF2 knockout mice.	28209760 (258)
	NRF2-KO vs NRF2-WT	High-fat plus fructose (HFFr) for 10 weeks, then additionally 5 nmol/g TBE-31 (acetylenic tricyclic bis (cyano enone)) for 6 weeks	TBE-31 treatment reversed insulin resistance in HFFr-fed wild-type mice, but not in HFFr-fed NRF2-null mice. TBE-31 treatment of HFFr-fed wild-type mice substantially decreased liver steatosis and expression of lipid synthesis genes, while increasing hepatic expression of fatty acid oxidation and lipoprotein assembly genes. Also, TBE-31 treatment decreased ER stress, expression of inflammation genes, and markers of apoptosis, fibrosis, and oxidative stress in the livers of HFFr-fed wild-type mice. By comparison, TBE-31 did not decrease steatosis, ER stress, lipogenesis, inflammation, fibrosis, or oxidative stress in livers of HFFr-fed NRF2-null mice.	29552625 (256)
	NRF2-KO vs NRF2-WT	LXRα agonist (T0901317) three times per week or every day for a week. SFN (90 mg/kg/day) for 2 days prior to T0901317 administration	NRF2-deficiency significantly enhanced fat accumulation in the liver in both experiments, liver weight gains were increased by T0901317 to a greater extent in NRF2-KO knockout mice, supporting that NRF2 antagonizes LXRα-induced lipogenesis. Sulforaphane suppressed T0901317-induced lipogenesis, as shown by a decrease in serum TG content and expression of lipogenic genes. NRF2 activation inhibits LXRα activity and LXRα-dependent liver steatosis by competing with FXR for p300, causing FXR activation and FXR-mediated SHP induction.	21504366 (236)
	Keap1-KD vs Keap1-WT	None	Reduced lipogenesis, lipid accumulation and hepatic steatosis. Improved glucose tolerance	24224011 (247)
	Keap1-KD vs Keap1-WT	Caloric restriction for 6 weeks	Increased fatty acid oxidation, reduced lipogenesis	23884569 (248)
	$Keap1^{\Delta hepa}$ (Hepatocytes specific KEAP1 knockdown mice)	MCD for 4 and 6 weeks and western diet for 13 weeks	Constitutive NRF2 activation in hepatocytes significantly improves catabolism (increased β-oxidation of fatty acids in mitochondria accompanied by marked repression of the lipogenic	26698665 (478)

(*continued on next page*)

Table 7 (*continued*)

Study object	Model	Treatment	Results	PMID
			transcription factor LXRα/β), strongly reduced lipogenic activity. Reduced accumulation of hepatic triglycerides in Keap1Δhepa mice in two experimental models of steatohepatitis.	
	Keap1$^{\Delta hepa}$/NEMO$^{\Delta hepa}$ vs NEMO$^{\Delta hepa}$ (Doble hepatocytes specific KEAP1and NEMO knockdown mice)	None	NEMO$^{\Delta hepa}$/KEAP$^{1\Delta hepa}$ livers showed reduced apoptosis compared to NEMO$^{\Delta hepa}$ livers as well as a dramatic downregulation of genes involved in cell cycle regulation and DNA replication. Consequently, NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ compared to NEMO$^{\Delta hepa}$ livers displayed decreased fibrogenesis, lower tumor incidence, reduced tumor number, and decreased tumor size.	33342543 (251)
	Adropin-KO	MCD and injection of AAV8-NRF2 or AAV8-GFP (tail vein) for 4 weeks	Adropin alleviated hepatocyte injury by upregulating the expression of Gclc, Gclm, and Gpx1 in a manner dependent on NRF2 transcriptional activity and by increasing the GSH levels. Adropin significantly increased CBP expression and promoted its binding with NRF2, which enhanced NRF2 transcriptional activity. Furthermore, AAV8-mediated overexpression of hepatic NRF2 expression functionally restored the liver injury induced by adropin-deficiency MCD-fed mice.	30684890 (249)
	Wild type	High-AGEs diet (cooked HF/HF during 120 °C for 20 min) and injection of AAV8-NRF2 or AAV8-GFP (tail vein) for 14 weeks	Induction of NRF2 reversed AGER1 downregulation, lowered the level of AGEs, and improved proinflammatory and fibrogenic responses in mice on a high-AGEs diet.	32657776 (250)
	Wild type	HFD or HFD-resveratrol (0.4 % (w/w)) for 16 weeks	Resveratrol attenuated HFD-induced methylation of the NRF2 promoter in the liver of mice, and this effect was correlated with reduction in triglyceride level and decrease in the expression of lipogenesis-related genes such as FAS and SREBP-1c.	31838177 (479)
	Wild type	MCD diet or MCD + i.p. rosmarinic acid from rosmary (10 mg/kg) for 8 weeks	Rosmaric acid decreased TG, cholesterol, ALT and AST in the plasma, decreased inflammation and hepatic steatosis, downregulated SREBP1c and FAS and upregulated PPARα, lowered NF-kB protein expression and decreased TNFα and IL6 expressions and reduced hepatic apoptosis	33787460 (480)
	Wild type	HF/HF or HF/HF + i.p. CORM-A1 (2 mg/Kg/day) for 7 weeks	CORM-A1 prevents hepatic steatosis in HF/HF diet, modulates KEAP1 expression, facilitates NRF2 translocation, and activates ARE genes in liver.	31514051 (481)
	Wild type	MCD- or MCD-S217879 (3 or 30 mg/kg/day) for 2 weeks. AMLN diet- or AMLN diet-S217879 (30 mg/kg) for 8 weeks	In MCD mice, disruption of KEAP1-NRF2 interaction with S217879 led to a dose-dependent reduction in MAFLD activity score while significantly increasing liver Nqo1 mRNA levels. In AMLN mice, S217879 treatment resulted in a significant improvement of established liver injury, with a clear reduction in both NAS and liver fibrosis. RNA-sequencing analyses revealed major alterations in the liver transcriptome in response to S217879, with activation of NRF2-dependent gene transcription and marked inhibition of key signaling pathways that drive disease progression.	36866391 (259)
	Wild type	STAM or control mice in the MASH inflammatory stage (week 8) with an i.p dose of vehicle or PHAR (50 mg/kg) for 5 days/week for 2 weeks.	MRI revealed that PHAR (disruptor of NRF2-β-TrCP interaction) protects against liver fat accumulation. Moreover, PHAR attenuated key markers of MASH progression, including liver steatosis, hepatocellular ballooning, inflammation, and fibrosis. Notably, transcriptomic data indicate that PHAR led to upregulation of 3 anti-fibrotic genes (Plg, Serpina1a, and Bmp7) and downregulation of 6 pro-fibrotic (including Acta2 and Col3a1), 11 extracellular matrix remodeling, and 8 inflammatory genes.	38184999 (260)
	Wild type	MCD diet or MCD-p.o. Silybin (105 mg/kg/day silybin) for 8 weeks	Silybin was effective in preventing the MCD-induced increases in hepatic steatosis, fibrosis and inflammation. The effect was related to alteration of lipid metabolism-related gene expression, activation of the NRF2 pathway and inhibition of the NF-κB signaling pathway in the MASH liver.	30191499 (253)
	Wild type	HFD or HFD-0.2 % and HFD-0.5 % JZE (w/w) for 10 weeks. MCD or MCD-p.o. JZE (200 and 500 mg/kg/day body weight) each day for 4 weeks.	JZE peel extract with an HFD or MCD ameliorated insulin resistance and improved lipid profiles, oxidative stress, hepatic steatosis and inflammation. JZE also prevented body weight gain and improved dyslipidemia in HF diet-induced mice. JZE improved lipid accumulation, oxidative stress and inflammation in the livers of the mice partly through NRF2 pathway. The hepatic expression level of NRF2 was significantly upregulated by 0.2 % JZE and 0.5 % JZE compared with that of the HFD group.	32559586 (254)
	Wild type	MCD diet or MCD-diet + p.o.ly isochlorogenic acid B (5, 10 and 20 mg/kg every day) for 4 weeks	ICAB significantly improved the pathological lesions of liver fibrosis. The levels of serum transaminases and plasma lipids were also significantly decreased by ICAB. In addition, ICAB inhibited hepatic stellate cells (HSCs) activation, and the expressions of hepatic genes involved in liver fibrosis. ICAB increased the levels of nuclear NRF2 protein.	30180301 (255)

expression levels of ATP-citrate lyase, the enzyme responsible for acetyl-CoA production, were increased in the liver of NRF2-null mouse compared to wild type pointing out NRF2 as a major regulator of cellular lipid disposition in the liver [235]. NRF2 plays a protective role against fat accumulation damage by limiting the lipogenic activity of liver X receptor α (LXRα). In fact, the hepatic steatosis induced by an LXRα agonist was increased or reduced by NRF2-deficiency or activation with SFN, respectively. The molecular connection has been explained thanks to the description of NRF2 as a repressor of LRXα activity. NRF2 competes for p300 deacetylation with Farnesoid X receptor (FXR), leading to the FXR-dependent induction of small heterodimer partner (SHP). SHP repression subsequently reduced LXRα-dependent gene transcription [236]. Accordingly, this relationship has been described in lipid and bile acid homeostasis in response to changes of cholesterol absorption. As expected, high cholesterol diet increased hepatic expression of liver X receptor α (LXRα) target genes related to fatty acid metabolism (*Fas*, *Acc1*, *Srebp-1c*, *Scd-1c* and *Cd36*), cholesterol transport (*Abcg5/abcg8*) and bile acid synthesis (*Cyp7a1*) in wild type mice. However, these genes were not induced in NRF2-null mice [237].

In general, MASH progression is worsened in NRF2-deficient mice compared to wild type mice, showing increased steatohepatitis, oxidative stress, and inflammation [238–240]. Hepatocyte- and macrophage-specific NRF2-KO mouse models have been used to elucidate the cell-specific role of NRF2 in the pathogenesis of MAFLD. Following a prolonged HFD exposure, NRF2 deletion in hepatocytes, but not in macrophages, attenuated hepatic steatosis. A possible mechanism is that NRF2 in hepatocytes is responsible for the expression of PPARγ to positively regulate lipid accumulation. Ablation of NRF2 in hepatocytes results in down-regulation of PPARγ expression and subsequent reduction of lipid accumulation [241].

Although MASH is usually associated with insulin resistance, HFD-fed NRF2-KO mice exhibited better insulin sensitivity than wild-type mice [240]. This could be partially dependent on the higher levels of FGF21 in plasma, liver and white adipose tissue of NRF2-KO compared to wild-type mice after prolonged HFD feeding [242]. To unravel the effects of prolonged non-canonical NRF2 activation in diabetes, a model of arsenic intoxication was used employing NRF2-KO, p62-KO, and wild-type mice. Integrated transcriptomic and metabolomic approach to assess diabetogenic changes in the livers evidenced that in contrast to canonical oxidative/electrophilic activation, prolonged non-canonical NRF2 activation via p62-mediated sequestration of KEAP1 increases carbohydrate flux through the polyol pathway, resulting in a pro-diabetic shift in glucose homeostasis. This p62-and NRF2-dependent increase in liver fructose metabolism and gluconeogenesis occurs through the upregulation of ketohexokinase (*Khk*), sorbitol dehydrogenase (*Sord*), triokinase/FMN cyclase (*Tkfc*), and hepatocyte nuclear factor 4 (*Hnf4A*) [243].

Likewise, methionine and choline-deficient (MCD) diets, with or without high fructose, are often used to induce MASH in mice. The NRF2 role in the evolution of MAFLD to MASH has been addressed comparing NRF2-KO and wild-type mice fed MCD diets. Livers from NRF2-null mice showed a substantial increase in macro- and microvesicular steatosis and a large increase in the number of neutrophils, compared to livers from wild-type mice treated similarly. Moreover, these livers suffered more oxidative stress than their wild-type counterparts as assessed by a significant depletion of reduced glutathione that was coupled with increases in oxidized glutathione and malondialdehyde. Furthermore, MCD diet-treated null mice suffered heightened inflammation as evidenced by activation of NF-κB-signature compared with livers from similarly treated wild-type mice [244].

Liver plasticity of the NRF2 system is evidenced by its capacity to be restored. Indeed, the administration of a diet-induced weight loss after HFD-feeding, restored gene expression of NRF2 and its target genes and is associated with reduced hepatic lipid accumulation and oxidative stress [245]. These results agree with the findings based on cholesterol supplementation which elevated NRF2 and hypoxia inducible factor α (HIF1α) expression, to stimulate liver regeneration, but if these proinflammatory signals persist, this profoundly alters hepatic metabolism and regeneration [246]. These findings are consistent with previous studies using KEAP1 knockdown mice in caloric restriction or in standard chow [247,248]. Furthermore, AAV8-mediated overexpression of hepatic NRF2 expression functionally restored liver injury induced by adropin-deficiency MCD-fed mice [249] and those fed a high-fructose diet (HFFr) [250].

In double KO mice, damage induced by chronic inflammation in liver has been studied. Hepatocyte-specific NEMO KO mice ($NEMO^{\Delta hepa}$) developed chronic liver disease with inflammation and steatohepatitis because of a missing regulatory subunit of the IκB kinase (IKK) complex in hepatocytes [251]. The condition seemed improved by NRF2 in a double mutant ($NEMO^{hepa}$/$KEAP1^{\Delta hepa}$) by reduced fibrogenesis as well as initiation and progression of hepatocellular carcinogenesis. This suggests that NRF2 activation upregulates genes involved in the antioxidant response, fatty acid oxidation and glucose metabolism and downregulating inflammatory and de novo lipogenesis genes [252].

Natural products have high potential in treating hepatic steatosis and inhibiting lipid-mediated oxidative stress by exerting antioxidant, lipid-lowering, anti-inflammatory, anti-fibrotic, and insulin-sensitizing effects [253–255]. Besides their potential to affect multiple aspects of liver steatosis disease, the safety and efficacy of natural products as NRF2 activators need to be carefully evaluated because they can vary depending on the specific dose, route of administration, and individual patient factors.

Pharmacological activation of NRF2 is a more controlled strategy to endorse protection. Indeed, the NRF2 activator TBE-31 reduces experimental MASH and liver fibrosis by inhibiting KEAP1 [256]. Similarly, CDDO-Im effectively prevented HFD-induced increases in body weight, adipose mass, and hepatic lipid accumulation in wild-type mice, but not in NRF2-disrupted mice. Moreover, the oxygen consumption and energy expenditure were higher in CDDO-Im than vehicle-treated mice. The levels of gene transcripts for fatty acid synthesis enzymes were downregulated after CDDO-Im treatment in the liver of wild-type mice. This inhibitory effect of CDDO-Im on lipogenic gene expression was significantly reduced in NRF2-disrupted mice [257]. In line, glucoraphanin supplementation of HFD attenuated weight gain, decreased hepatic steatosis, and improved glucose tolerance and insulin sensitivity in HFD-fed wild-type mice, but not in HFD-fed NRF2-KO mice [258]. Although electrophilic KEAP1 inhibitors mitigate MASH in diet-fed mice they can affect other proteins, causing side effects. Thus, new KEAP1 inhibitors are being developed to non-covalently disrupt the KEAP1-NRF2 complex. One such compound, S217879, has shown beneficial effects on MASH and liver fibrosis but can cause hepatomegaly [259]. Additionally, strong NRF2 activation from KEAP1 interface mutations is found in many tumors, including hepatocellular carcinomas. Clinical data also suggest caution with excessive NRF2 activation due to potential liver issues in treated patients. In this regard, activation of NRF2 within the physiological range by PHAR, novel disruptor of NRF2-β-TrCP interaction, attenuated key markers of MASH progression, including liver steatosis, hepatocellular ballooning, inflammation, and fibrosis. Notably, transcriptomic data indicate that PHAR led to upregulation of 3 anti-fibrotic genes (*Plg*, *Serpina1a*, and *Bmp7*) and downregulation of 6 pro-fibrotic (including *Acta2* and *Col3a1*), 11 extracellular matrix remodeling, and 8 inflammatory genes [260].

MAFLD and T2DM commonly coexist and act synergistically to drive adverse clinical outcomes. Nigro et al. [261], showed the effects of the specific contribution of both saturated fats and fructose, on the progression of MAFLD and insulin resistance. Focusing on the analysis of NLRP3 inflammasome and KEAP1-NRF2 antioxidant signaling pathways in the liver of mice fed a standard, a saturated fat (HFAT) or a fructose (HFRT) diet for 12 weeks, the authors reported how, despite lower caloric intake, the HFRT led to a more severe steatosis and more pronounced pro-oxidant and proinflammatory signaling activation with

respect to the HFD. An increased levels of cytosolic NRF2 and of its inhibitor KEAP1, paralleled by impaired NRF2 nuclear translocation in the HFRT group, were associated with a greater activation of the SREBP lipogenic pathway thus indicating that saturated fats and fructose in the diet exert dissimilar effects on liver lipid metabolism. Indeed, fructose has a higher lipogenic power than fats, being both SREBP1c and SREBP2 pathways activated to a greater extent in the liver of fructose-fed mice compared to fat-fed mice. Since a decrease in NRF2 activity in hepatic steatosis could promote the progression of MAFLD, the anti-lipogenic effect exerted by NRF2 through inhibition of LXRα, a master regulator of lipid homeostasis [236], provide the potential for using NRF2 activators for the treatment of MAFLD.

Although loss of NRF2 increases sensitivity to MAFLD, it is less certain whether upregulation of NRF2 by genetic or pharmacologic approaches decreases sensitivity to the disease. Treating MAFLD with diabetic drugs, exercise and/or natural products regarding lipid metabolism, oxidative stress, and inflammation is a promising treatment prospect. Activating NRF2 after the onset of diabetes might reverse insulin resistance along with advanced stages of MAFLD. Zhang et al. showed that upregulation of the AMPK-NRF2-HO-1 signaling pathway might be the key mechanism of metformin and exercise synergistic effect, which may have additive effects on alleviating hepatic lipid accumulation [262]. Empagliflozin is an SGLT-2 inhibitor recently reported to exert glycemic as well as pleiotropic effects on the progression of MAFLD and its symptoms [263]. In a non-obese prediabetic rat model, Hüttl et al. [264] reported the beneficial effect of empagliflozin against the MAFLD development by stimulating antioxidant enzyme activity, SOD, and GPx via upregulation of NRF2. Using a genetic mouse model ($Spta^{sph/sph}$) of spherocytosis and single-cell RNA sequencing, Pfefferlé et al. [265], discovered that hemolysis transforms liver macrophages into anti-inflammatory erythrophagocytosis sustained by activation of a heme/NRF2 signaling pathway. These anti-inflammatory macrophages mitigated disease expression in a MAFLD model induced in ($Spta^{sph/sph}$) mice by feeding them MCD diet. The authors reported that the NRF2-activating drug DMF mimicked the effect of chronic hemolysis on MAFLD progression in the MCD model thus demonstrating the role of heme-induced NRF2 signaling in the regulation of inflammatory phagocyte functions. Despite belonging to a relatively new class of pharmaceuticals, increasing evidence demonstrates biological drugs have promising potential to treat MAFLD. By *in vivo* studies, Lin et al. [266] reported that the treatment with recombinant fibroblast growth factor 1 (rFGF1) in diabetes associated MAFLD mouse model (HF/high sucrose diet, HFHS) stimulated NRF2 and antioxidant protein expression via activation of hepatic AMPK. The role of CLUSTERIN, a multi-functional protein that is up regulated in the pathogenesis of various metabolic diseases, including MAFLD, has been investigated by Park et al. [267] by using transgenic mice with whole-body clusterin overexpression (wCLU-tg) in Western diet-induced MAFLD. The authors reported that clusterin by activating NRF2 and AMPK, protects against Western diet induced MAFLD and opens the way to its translation in preclinical studies as a biological drug. Moreover, Zai et al. [268] showed a new promising therapeutic strategy based on nanoparticles for liver-targeting IL-22 gene delivery to be utilized for alleviating obesity-related fatty liver via NRF2 activation. Of note, in a murine model of steatosis, Ferrigno et al. [269] reported for the first time the beneficial effects of metabotropic glutamate receptor (mGluR)5 antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP). MPEP induces a reduction of lipid accumulation and oxidative stress in obese (ob/ob) mice fed with HFD for 7 weeks. This result confirms the administration of MPEP produces anti-inflammatory and antioxidative effects in this model of hepatic steatosis by decreasing the mRNA expression of both NF-κB and NRF2. However, the underlying mechanisms by which mGluR5 antagonist contributes to prevent MAFLD need to be further investigated. Collectively, these findings form the basis of a novel complementary therapeutic strategy for MAFLD targeting NRF2 activation to restore injured liver tissue and attenuate oxidative stress.

4.4.6. Mouse models of muscular dystrophies

In skeletal muscles, ROS management is particularly relevant. The levels of ROS fluctuate in line with the intensity of muscle contraction, which is directly linked to mitochondrial activity, and mediate a series of physiological adaptations. Apart from the superoxide anions generated by the mitochondria, labile iron is also an important element in oxidative stress signaling in the muscle, with approximately 10–15 % of total iron or heme-iron of the body found in skeletal muscle. The importance of heme-iron in the correct functioning of the skeletal muscle is further illustrated by the fact that heme is a co-factor of key cellular components, including myoglobin and cytochrome *c* oxidase. Under pathological conditions, ROS accumulation in the muscle leads to mitochondrial dysfunction and muscle atrophy, a common feature in several muscular dystrophies, a group of diseases characterized by progressive weakness and loss of muscle mass [270–273]. Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy diagnosed during childhood and is characterized by mutations in *DMD*, which encodes the cytoskeleton-transmembrane receptor bridging protein, dystrophin [274]. As for other muscular dystrophies, increased oxidative stress [275,276], iron overload [277], as well as chronic inflammation [278,279] are hallmarks of DMD and therefore antioxidant mechanisms led by NRF2, have been the focus of intense research [280,281]. These studies have shed light on the importance of NRF2 and downstream targets to counter the oxidative stress linked to muscular dystrophies (Table 8). In particular, the crosstalk between increased ROS and inflammatory response has been shown to occur both in patients and in *mdx* mice [281], the preferred mouse model for DMD. This crosstalk is likely mediated by NF-κB activation in response to mechanical stress and related production of ROS, as shown in previous studies using the *mdx* mouse model [282]. In accordance, NRF2 induction dampens NF-κB activation, consequently reducing the inflammatory response [283]. Further supporting a direct role of NRF2 in countering disease pathology in the context of muscular dystrophies, deletion of *Nrf2* in the natural A/J mouse model of dysferlinopathy, associated with mutations in *DYSF*, led to a dramatic increase in disease pathology [284]. NRF2 has also been shown to play a role in skeletal muscle regeneration. Absence of NRF2 in a model of hindlimb ischemia-reperfusion injury (*i.e.* muscle injury), did not worsen the extent of the injury, but led to an impairment in muscle regeneration, which is likely associated with the transcriptional modulation of key myogenic factors and consequently to the regulation of muscle stem cell proliferation and differentiation [285]. The role of NRF2 in skeletal muscle physiology has also been tested in the context of exercise, where *Keap1* knockdown mice (*Keap1*-KD) had increased exercise endurance [286]. Moreover, different studies have also shown that *Nfe2l2* deletion worsens the muscle ageing phenotype [287–289]. Together these findings support the idea that inducing antioxidant mechanisms may be the key to counter the overall disease pathology in muscular dystrophies. In keeping with this notion, several studies have tested the ability of classic antioxidant compounds, such as NAC to revert disease pathology in *mdx* mice [290–292]. In addition to DMD, treatment with NAC has also been used to evaluate the effectiveness of antioxidant treatment in countering other types of muscular dystrophies. This is true for *LAMA2* congenital muscular dystrophy (LAMA2-CMD), a disease caused by mutations in *LAMA2*, encoding the α2 chain of laminins 211 and 221 [272,293]. NAC treatment was shown to counter muscle weakness, prevent the erroneous central nucleation of muscle fibers and decrease apoptosis, inflammation, fibrosis and oxidative stress [293]. NAC treatment was also shown to allow muscle regeneration upon injury in $Stra13^{-/-}$ mice, which lack the basic helix-loop-helix transcription factor *Stra13* (or *Bhlhe40*), known to play an important role in muscle development [294]. Contrasting with this beneficial role of NAC in the treatment of muscular dystrophies, some studies have also highlighted NAC side effects, including a negative impact on body weight gain and gain of muscle mass and a decrease in the liver weight [291,295] thus supporting the use of other strategies based on the activation of endogenous

Table 8
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in muscle related diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are models in which NRF2 has been stimulated employing direct strategies using classical activators (blue) or indirect methods (grey). *Dimethyl fumarate (DMF); sulforaphane (SFN); oral administration (p.o.).*

Study object	Model	Treatment	Results	PMID
Duchenne muscular dystrophy	*mdx*	2 mg/kg body weight, p.o. gavage, 4 weeks treatment SFN	Improved muscle mass, promoted sarcolemma integrity, and reduced inflammation	26013831 (255)
	mdx	2 mg/kg body weight, p.o. gavage, 8 weeks treatment SFN	Protected dystrophic muscle from oxidative damage, improved muscle function and pathology, and improved myocardial hypertrophy	25593219 (296)
	mdx	2 mg/kg body weight, p.o. gavage, 12 weeks treatment SFN	Reduced skeletal and cardiac muscle fibrosis and diminished inflammatory response	26494449 (297)
	mdx	100 mg/kg body weight, p.o. gavage, daily for 2 weeks DMF	Improved muscle function, activation of antioxidant and anti-inflammatory responses and increased mitochondrial respiration	37751291 (482)
	mdx	1 mg/kg body weight, p.o. gavage, once a day for 4 weeks (−)-Epicatechin	Improved dystrophin-associated protein complex expression and localization in the frontal cortex	28855126 (298)
	mdx	0.04, 0.4, or 4 g/kg food (estimated to be 5, 50, or 500 mg/kg body weight/day, respectively) administered ad libitum for 56 weeks Resveratrol	Improved mitophagy defect and cardiomyopathy	30348945 (304)
	mdx	4 g/kg food administered ad libitum for 32 weeks Resveratrol	Countered muscle mass loss and decreased oxidative damage	21652783 (302)
	mdx	0.04, 0.4, or 4 g/kg food administered ad libitum Resveratrol for 56 weeks	Improved mitophagy defect, reduced the number of centrally nucleated muscle fibers and improved muscle function	30510631 (303)
	mdx	0.1 mg/kg, 0.5 mg/kg or 1 mg/kg body weight, for 10 days Curcumin	Ameliorated sarcolemma integrity and muscle strength	18460899 (483)
	mdx	3000 μg/mL in RO drinking water, for 6 weeks Adenylosuccinic acid	Ameliorated dystrophic muscle pathology and oxidative damage, but not muscle contractibility	31980663 (484)
	mdx	100 mg/kg body weight, p.o. gavage, daily for 2 weeks Cilostazol	Reduced ROS levels and lipid peroxidation and decreased apoptosis	36565167 (485)
	mdx	200 mg/kg body weight, p.o. gavage, daily for 9 months idebenone	Prevented diastolic dysfunction and cardiac failure, reduced cardiac fibrosis and inflammation and improved muscle function	18784063 (486)
	Myf5$^{Cre/+}$, *Fktn* $^{L/L}$	2 mg/kg body weight, p.o. gavage, once a day for 4 weeks Rapamycin	Reduced fibrosis and inflammation, decreased activity-induced damage, and reduction in the central nucleation and increased size of muscle fibers	27257474 (307)
	mdx	1 ml nanoparticles emulsion/kg body weight (approximately ~0.002 mg rapamycin/animal/ treatment), two times a week for 4 weeks	Increased skeletal muscle strength and cardiac contractility	24500923 (306)
	mdx	1 mg rapamycin beads per injection, followed by a 0.5 mg rapamycin beads per injection 2 weeks later	Reduced muscle fiber necrosis and increased muscle regeneration	24500923 (306)
LGMD	δ-KO (B6.129-Sgcdtm1Mcn/J)	1 mg/kg body weight, p.o. gavage, twice a day for 14 days ' (−)-Epicatechin	Improved antioxidant response, decreased apoptosis, reduced fibrosis and enhance muscle function	25284161 (299)
Muscular dystrophy	*Lmna*$^{-/-}$	Diet containing encapsulated rapamycin, ad libitum starting at 3–4 weeks of age and present throughout lifespan	Improved cardiac and skeletal muscle function and increased survival	22837538 (309)
Muscle injury	CD-1 with BaCl2-induced damage	1 mg/kg body weight, p.o. gavage, twice a day up to 14 days (−)-Epicatechin	Promoted muscle repair upon injury and expression of myogenic factors	35609849 (301)

mechanisms. Nevertheless, and considering the undoubtful role of ROS in the pathology of muscular dystrophies, *mdx* mice have been used as proof of concept to test possible therapeutic strategies aimed at directly activating NRF2 to ameliorate DMD disease pathology. For example, administration of SFN has been shown to improve the pathology of dystrophic muscles, leading to an increase in muscle strength and mass and reduced inflammation and fibrosis [283,296,297]. (−)-Epicatechin, a flavonoid that activates NRF2 pathway, improved dystrophin-associated protein complex expression and localization in the frontal cortex of *mdx* mice [298]. The use of this same compound has been also evaluated in other models of muscle dystrophies, including *δ*-KO [299], a model for limb girdle muscular dystrophies (LGMD), a group of disorders characterized by weakness and atrophy of the limb and shoulder girdle muscles and that is linked to mutations in different genes, such as *LMNA* (encoding LAMIN A), *CAPN3* (encoding calpain 3), *DYSF* (encoding dysferlin) and *SGCA-D* (encoding sarcoglycan complex components A-D) [300]. (−)-Epicatechin treatment of *δ-KO* mice enhanced overall muscle function, possibly by contributing to improve antioxidant response, to decrease apoptosis and to reduce fibrosis [299]. The positive effect of (−)-epicatechin was also shown in a model of $BaCl_2$-induced muscle damage, where the improvement on muscle repair was also evident [301]. Another NRF2 activator, resveratrol, has been shown to improve muscle mass gain, counter oxidative damage, reduce cardiomyopathy and restore mitophagy in *mdx* mice [302–305]. The mTOR inhibitor and activator of the NRF2 pathway, rapamycin, has also been used to ameliorate the pathophysiology in different models of muscular dystrophy [306,307]. For example, for DMD the treatment of *mdx* mice with rapamycin led to a reduction in muscle fiber necrosis, increased muscle regeneration, increase in skeletal muscle strength and cardiac contractility [306,308]. *Myf5*$^{Cre/+}$, *Fktn*$^{L/L}$ is a fukutin-deficient mouse model of dystroglycanopathy, also known as Fukuyama muscular dystrophy. This model allows the conditional deletion of the *Fktn* gene, encoding the transmembrane protein fukutin, which locates to the Golgi and is involved in the glycosylation of different proteins, possibly including α-dystroglycan. Deletion of *Fktn* only occurs in cells that express *Myf5*, an important marker for muscle development, where Cre recombinase is conditionally expressed. Rapamycin treatment of *Myf5*$^{Cre/+}$, *Fktn*$^{L/L}$ mice allowed to reduce the levels of fibrosis and inflammation, decreased activity-induced damage, and led to a reduction in the central nucleation and increased size of muscle fibers [307]. In *Lmna*$^{-/-}$ mice, which lack LAMIN A/C, an important protein to maintain nuclear structure, rapamycin treatment improved cardiac and skeletal muscle function and increased survival [309]. The specific contribution of NRF2 to rapamycin beneficial outcomes should be

addressed in future studies.

Taken together, these studies support that targeting NRF2 pathway seems to be a central avenue for treatment of muscular dystrophies. Accordingly, some of these compounds have already been tested in patients with promising results, as reviewed previously for DMD [310], highlighting the potential use of Idebenone, coenzyme Q10 and flavonoids. Similarly for other muscular dystrophies such as Becker muscular dystrophy, the use of (−)-epicatechin in patients has been shown to improve muscle pathology and mitochondria biogenesis [311]. Resveratrol is yet another compound that has been on a phase II clinical trial in patients with DMD, Becker muscular dystrophy or Fukuyama muscular dystrophy [312].

4.4.7. *Mouse models for neuropsychiatric conditions*

Depression is a mental disorder characterized by persistent low mood, loss of interest, feeling of sadness, difficulty in concentrating or making decisions, cognitive impairment, sleep changes, decreased appetite, and suicidal tendencies, which severely limits the patients' psychosocial function and quality of life [313].

Many of the factors that influence depression such as physical inactivity, cardiovascular disease, cancer, diabetes or respiratory diseases can contribute to depressive behavior. In turn, people with these diseases may also find themselves experiencing depression due to the difficulties associated with managing their condition. Different hypotheses have emerged throughout the years to try to explain the pathomechanisms implicated in depression. One of the most longstanding has been the monoaminergic hypothesis proposed by Schildkraut in 1965 [314]; this hypothesis is sustained on a reduction of brain levels of adrenaline, dopamine, and serotonin. In fact, most treatments used today for the treatment of depression such as serotonin or norepinephrine reuptake inhibitors and monoamine oxidase inhibitors (MAOI) align with this hypothesis. However, these drugs can take months to show an effect and have multiple side effects. Furthermore, these drugs show no efficacy in around 30–40 % of the patients. Therefore, other mechanisms must be involved in the pathogenesis of depression. Among these other mechanisms, inflammation, oxidative stress, reduced neurotrophic factors and activation of the hypothalamic-pituitary-adrenal (HPA) axis (stress, increased cortisol) have been proposed as contributors that are known to be interconnected. For example, peripheral blood inflammatory biomarkers can access the brain and interact with virtually every pathophysiologic domain known to be involved in depression, including neurotransmitter metabolism, neuroendocrine function, and neural plasticity. Indeed, activation of inflammatory pathways within the brain is believed to contribute to decreased neurotrophic support and altered glutamate release/reuptake, as well as oxidative stress, leading to excitotoxicity and loss of glial elements, consistent with neuropathologic findings that characterize depressive disorders [315].

In this respect, NRF2 is being proposed as an innovative target to develop drugs for the treatment of depression as its induction would induce genes implicated in the regulation of neuroinflammation, oxidative stress, mitochondrial dysfunction, autophagy and ferroptosis (Table 9). Results obtained from NRF2-KO mice show that the absence of NRF2 causes a depressive-like behavior indicated by an increase in the immobility time in the forced swimming test and the tail suspension test, and by a reduction in the grooming time in the sucrose splash test (anhedonia). In brain tissue of NRF2-KO mice microgliosis, astrogliosis, reduction of serotonin and dopamine levels, increased glutamate levels, together with altered levels of the neurotrophic factor BDNF have been reported [316,317]. Taken together, NRF2-null mice recapitulate most of the hallmarks that characterize a depressive phenotype.

Depressive phenotypes can be modelled in animals by inducing a neuroinflammatory response by injecting LPS, by chronic corticosterone administration or by subjecting them to social defeat or chronic mild stress paradigms. In the LPS model, administration of the NRF2 inducer SFN prevented the depressive phenotype shown in the NRF2-KO animals [316]. HPA axis dysfunction and increased cortisol levels have long been associated with Major Depressive Disorder (MDD) [318]. Based on this observation, chronic treatment with corticosterone produced a depressive phenotype in mice. In this model, the endogenous compound agmatine reverted the behavioral depressive phenotype by a mechanism that implicated the induction of NRF2. Agmatine increased the expression of NRF2 target genes and its protective effect in the corticosterone model was prevented in NRF2-KO mice [319]. In a chronic social defeat stress (CSDS) model, edaravone exhibited a potent antidepressant and anxiolytic properties through SIRT1/NRF2/HO-1/GPX4 axis [320]. These observations, summarized in Table 9, indicate that NRF2 induction could provide a beneficial effect for depressive conditions. Although, there is preclinical and clinical evidence indicating that NRF2 is lower in depression [321], there is still no drug approved for the treatment of depression based on NRF2 induction. At this moment a clinical study using SFN for the treatment of mild to moderate depression in patients with history of cardiac interventions has shown non-conclusive results [322].

Schizophrenia is a chronic and highly complex psychiatric disorder characterized by cognitive dysfunctions, negative symptoms including decreased emotion, delusions and hallucinations. In recent years, the deficiency of glutamate neurotransmitter system has been paid more attention in the pathogenesis of schizophrenia, which suggested that abnormal concentration of Glu or GABA in the brain of schizophrenia patients is closely related to schizophrenia [323]. Recently, it has been estimated that over 20 different animal models of schizophrenia have been developed [324], all fit into four different induction categories: developmental, drug-induced, lesion or genetic manipulation.

Specifically, the NRF2 pathway has been investigated in schizophrenia employing mainly the drug-induced models. Blockade of the N-methyl-D-aspartate (NMDA) receptor by non-competitive antagonists such as phencyclidine (PCP), induces delusions and hallucinations in otherwise healthy subjects, symptoms commonly seen in schizophrenia. Acute PCP administration causes hyperlocomotion, social withdrawal, and impairment of both prepulse inhibition of acoustic startle and cognition in rodents [325]. Remarkably, PCP administration in mice has been associated with increased NRF2 nuclear translocation and DNA binding activity. Indeed, the mRNA levels of *Gclcm* and *Gclc* were also elevated in the prefrontal cortex of the PCP-treated mice compared to control [326]. On the other hand, it has been reported a down-regulation of NRF2 signaling in PCP-induced mouse model of schizophrenia, as evidenced by decreased expression of NRF2, HO-1 and NQO-1 but following sub-chronic PCP treatment [327]. In a similar model, pretreatment with SFN attenuated PCP-induced cognitive deficits, oxidative stress, reduction of spine density and decrease in parvalbumin (PV) -positive cells in the medial prefrontal cortex and hippocampus of mice. In addition, intake of glucosinolate SFN precursor glucoraphanin, during the juvenile and adolescence prevented the onset of cognitive deficits, oxidative stress and loss of PV immunoreactivity induced by PCP treatment [328].

Emerging epidemiologic data state that gestational exposure to infection contributes to the etiology of schizophrenia [329]. Maternal immune activation (MIA) using polyriboinosinic-polyribocytidilic acid [poly (I:C)], a Toll-like receptor 3 agonist, has been widely used as a neurodevelopmental animal model for schizophrenia. The role of NRF2 has been addressed evaluating schizophrenia hallmarks after its activation. Dietary intake of glucoraphanin during pregnancy and lactation prevented cognitive and social interaction deficits, as well as reduction of PV immunoreactivity, in the medial prefrontal cortex of the juvenile and adult offspring after MIA [330]. The effects of another activator of NRF2, DMF have been investigated in a study which also used prenatal immune activation model of schizophrenia in mice by maternal injection of the viral mimetic poly (I:C) during pregnancy. Prolonged DMF treatment of the male mice offspring counteracted the changes in the medial prefrontal cortex structure and behavior induced by MIA [331]. Together these studies correlate a protective role of NRF2 activation with a reduction of schizophrenia outcomes, however future studies are

Table 9

Examples of studies using *M musculus* as model organism to examine the role of NRF2 in neuropsychiatric diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null expression models for NRF2 (pink), models employing NRF2 overexpression (green), and those in which NRF2 has been stimulated employing direct strategies using classical activators (blue) or indirect methods (grey). *Dimethyl fumarate (DMF); kainic acid (KA); N-acetyl cysteine (NAC); intraperitoneal injection (i.p.); intraventricular injection (i.c.v.); oral administration (p.o.); phencyclidine (PCP); phenytoin (PHT); sulforaphane (SFN); subcutaneous administration (s.c.); 4-Octyl itaconate (4-OI).*

Study object	Model	Treatment	Results	PMID
Depression	NRF2-WT and NRF2-KO	Rofecoxib, (i.p. 2 mg/Kg/day) or SFN (i.p. 1 mg/kg/day) for 1 week and LPS (i.p. 0.1 mg/kg) for 24h	NRF2 deficient mice exhibit depressive-like behavior, and that this phenotype is reversed by an anti-inflammatory drug such as rofecoxib or SFN in wild type littermates.	23623252 (316)
	NRF2-WT and NRF2-KO	Agmatine (p.o. 0.1 mg/kg/day) in a depressive-Like model induced by chronic administration of corticosterone for 21 days.	Agmatine treatment for 21 days was able to abolish the corticosterone-induced depressive-like behavior and brain alterations in an NRF2-dependent manner.	25966970 (319)
	Wild type	Edaravone (i.p. 10 mg/kg/day) for 10 days	Edaravone possesses potent antidepressant and anxiolytic properties in a chronic social defeat stress model through SIRT1/NRF2/HO-1/Gpx4	35130906 (320)
Schizophrenia	Wild type	Phencyclidine (s.c. 10 mg/Kg/day) for 2 weeks. Mountain-cultivated ginseng post-treatment (i.p. 200 mg/kg/day) for 3 weeks	PCP induces NRF2 nuclear accumulation, that was even higher when post-treated with mountain-cultivated ginseng. The expression levels of *Gclm* and *Gclc* were also elevated in prefrontal cortex of the PCP-or PCP-ginseng-treated mice compared to control.	27668757 (326)
	Wild type	Phencyclidine (s.c. 10 mg/Kg/day) for 10 days. Andrographolide post-treatment (p.o. 5 or 10 mg/kg/day) for 7 days. ML385 post-treatment (p.o. 30 mg/kg/day) for 7 days.	Andrographolide increased expression of NRF-2, HO-1 and NQO-1, promoted nuclear translocation of NRF-2 through blocking the interaction between NRF-2/KEAP1. ML385 abrogated andrographolide-mediated improvement in PCP model.	34294378 (327)
	Wild type	SFN (i.p. 30 mg/kg/day) and/or PCP (s.c. 10 mg/Kg/day) for 10 days.	SFN attenuated PCP-induced cognitive deficits, oxidative stress, reduction of spine density and decrease in parvalbumin (PV) -positive cells in the medial prefrontal cortex and hippocampus	26107664 (328)
	Wild type	SFN (i.p. 3, 10, and 30 mg/kg) PCP (s.c. 3.0 mg/kg)	SFN (only at 30 mg/kg) significantly attenuated hyperlocomotion in mice after single PCP administration.	23430731 (487)
	Wild type	poly (I:C) (i.p. 5.0 mg/kg/day) for six days (E12-E17). 0.1 % glucoraphanin in food pellets for 28 days (P28–P56).	Glucoraphanin prevented the onset of cognitive deficits, oxidative stress and loss of PV immunoreactivity in the medial prefrontal cortex of adult mice	29391571 (488)
	Wild type	poly (I:C) (i.p. 5.0 mg/kg/day) for six days (E12-E17). 0.1 % glucoraphanin in food pellets for 26 days (E5-P21).	Glucoraphanin prevented cognitive and social interaction deficits, and reduction of PV immunoreactivity in the medial prefrontal cortex of the juvenile and adult offspring	32463181 (488)
	Wild type	poly (I:C) (i.p. 5.0 mg/kg/day) at day E9.5. DMF (i.p. 50 mg/kg/day) for 14 days	DMF counteracted the changes in medial prefrontal cortex structure (decrease in dendritic complexity and spine density) and behavior (abnormal exploratory and depression-related behavior)	34530042 (331)
Autism	NRF2-KO vs NRF2-WT	Valproic acid (s.c. 400 mg/kg) (P14)	NRF2-KO mice perform similarly to wild type mice in developmental behavior. assays but were more sensitive to Valproic acid as seen in the rotarod and water maze.	25454122 (333)
	BTBR *T*$^{+}$*_Itpr3tf/J*	SFN (i.p. 50 mg/kg/day) for 1 week	SFN induced lower self-grooming/marble burying behavior, higher social interaction in 3 chambered sociability test, reduced Th17 immune responses and oxidative stress parameters, upregulated enzymatic antioxidant defenses in neutrophils/cerebellum	30790585 (335)
	BTBR *T*$^{+}$*_Itpr3tf/J*	1 mg/kg/day Se (p.o. Na2SeO3, equivalent to 0.45 mg of elemental Se/kg/day) for 4 weeks. 30 mg/kg ML385 (i.p. 30 mg/Kg) once every other day for 4 weeks	Selenium ameliorated impairments in learning and memory, improved social functions, decreased repetitive behaviors, inhibited ferroptosis and increased levels of NRF2 and GPX4 in hippocampus. ML385 reduced effect of selenium on ferroptosis and abnormal behaviors	35227767 (334)
Epilepsy	ARE–hPAP; NRF2-KO vs NRF2-WT	Kainic acid (i.p. 25 mg/Kg)	ARE-hPAP activation was noted in the CA1 and CA3 hippocampal regions after KA injection, mainly in astrocytes. NRF2-KO mice evidenced elevated seizure severity and duration, hippocampal neuron damage and mortality, altered GFAP immunoreactivity, increased microglial infiltration compared to wild type.	16945104 (340)
	NRF2-KO vs. NRF2-WT	SFN (i.p. 5 mg/kg/day) at 30 min before every electric stimulation for amygdala kindling.	SFN in hippocampus suppressed the progression of amygdala kindling and ameliorated the cognitive impairment and oxidative stress induced by epileptic seizure. This response was hindered in NRF2-ko mice.	24333359 (344)
	Wild type	AAV-hNRF2 (i.c.v. in CA4 region) or AAV-CTRL 3 weeks after pilocarpine (i.p.)	*Nf2el2*, *Hmox1*, *Nqo1* and *mGst* expression levels increased progressively in mice, reaching a peak at 72 h after spontaneous seizures, and then declined. Mice injected with AAV-hNRF2 displayed significantly fewer generalized seizures, with profound reduction in microglia activation. Hippocampal neurons were preserved, whereas the number of astrocytes was unchanged.	23686862 (338)
	Wild type	Gintonin (p.o. 100 mg/kg) was administered 2 h before Kainic acid (0.2 μg, i.c.v.) injection.	The expression of NRF2, NQO1 and HO-1 was slightly but significantly upregulated in the hippocampus after kainate. This upregulation was not evidenced in gintonin treated	37252272 (341)

(*continued on next page*)

Table 9 (*continued*)

Study object	Model	Treatment	Results	PMID
			group. Gintonin stimulates NRF2 signaling in the hippocampus affected by KA i.c.v. injection. Pretreatment with gintonin protected against seizure and hippocampal cell death induced by kainate.	
	Wild type	Quercetin (p.o. 50 mg/kg/day) for 21 days before Kainic acid (i.p 20 mg/Kg)	Quercetin alleviated seizure-like behavior, learning and memory deficits, reversed decrease in NRF2, SLC7A11, and GPX4 levels	36087883 (339)
	Wild type	MP (i.p. 38 mg/kg/day) for 23 days to induce seizures. PHT (i.p. 24 mg/kg) administered at day 24. NAC (i.p. 70 mg/kg/day) before MP and/or 4-OI (i.p. 30 mg/kg/day) were injected 1 h after MP stimulation for 23 days	Antioxidative preventive treatment with NAC prevented the mice from entering a PHT-resistant state. PHT resistance could be achieve also by activating the NRF2/P-gp pathway with 4-OI.	36028602 (343)
	Wild type	SFN (i.p. 5 mg/Kg/day) for 5 days prior to 6 Hz seizure stimulation or pilocarpine (s.c. 360 mg/Kg)	SFN elevated seizure threshold	26365487 (489)
	Wild type	SFN (i.p. 10, 50, 100 and 200 mg/kg)	SFN at toxic dose injected acutely exerted proconvulsant activity	28412310 (490)

needed to establish a causality link.

Autism, also called autism spectrum disorder, is a neurodevelopmental disorder marked by deficits in reciprocal social communication and the presence of restricted and repetitive patterns of behavior. Autism symptoms, including impairments in language development, social interactions, and motor skills, have been difficult to model in rodents. The fact that children exposed in utero to sodium valproate (VPA), clinically in adults as an anticonvulsant agent, demonstrate behavioral and neuroanatomical abnormalities like those seen in autism paves the way to the generation of a novel model to mimic autism in mice. Indeed, mice exposed to valproic acid on embryonic or early postnatal exhibit developmental behavioral deficits like those observed in autism as well as enhanced neuronal death in the cerebellum and hippocampus, regions known to be affected in humans with autism [332]. Recent studies demonstrated that NRF2 might be linked to autism through its antioxidant and anti-inflammatory effects. Indeed, NRF2 null mice exposed to early postnatal treatment with VPA had behavioral deficits in open field and rotarod activity, as well as in learning and memory in the Morris water maze, in comparison to wild type mice, suggesting increased sensitivity of NRF2-KO mice to the valproic acid-induced neural damage [333].

Selenium treatment of BTBR (BTBR *T* + *Itpr3tf/J*) mice, used to model autism spectrum disorders, ameliorated impairments in learning and memory, improved social functions, decreased repetitive behaviors, inhibited ferroptosis and increased levels of NRF2 and GPX4 in the hippocampus, whereas treatment with the protein synthesis inhibitor ML385 reduced the effect of selenium on ferroptosis and abnormal behaviors in BTBR mice [334]. Further evidence suggested that activation of NRF2 by SFN exerts beneficial effects on immune dysfunction and oxidative imbalance in both periphery and brain of BTBR mice, resulting in the ameliorated autism-like symptoms [335]. In the same BTBR mouse model of autism, toxic environmental pollutants, di-2-ethylhexyl phthalate (DEHP) [336], as well as methylmercury [337], caused worsening of autism-like symptoms, related to a lack of NRF2 signaling pathway upregulation as demonstrated by unaltered expression of NRF2, HO-1, and SOD-1 in the periphery and cortex.

Mesial temporal lobe epilepsy (MTLE) is the most common type of partial epilepsy in adults is a chronic neurological disorder that in a sizable population of patients is associated with progressive neuronal damage and poor control of seizures by currently available treatments. Numerous alterations have been reported in patients with MTLE, including hippocampal sclerosis and cell death, mossy fiber sprouting, reorganization of hippocampal interneuronal networks, gliosis, blood-brain-barrier dysfunction and angiogenesis. Employing transcription factor enrichment analysis (TFEA) on publicly available gene, expression data sets from a variety of epilepsy-related studies identified NRF2 as a potential therapeutic candidate [338]. Moreover, it has been demonstrated that the NRF2-mediated ferroptosis pathway may be associated with the occurrence of seizures in a clinical setting. The mRNA expression levels of *NFE2L2*, *SLC7A11*, and *GPX4* were significantly reduced in peripheral blood from patients with newly diagnosed and untreated seizures [339].

In mice the most common drug to mimic MSTLE is kainate, a cyclic analog of L-glutamate and an agonist of the ionotropic kainic receptors. Analysis of transgenic reporter mice, ARE-hPAP, following kainate injection revealed selective reporter activation within the damaged hippocampus. Relevantly, NRF2-KO mice were shown to be more sensitive to kainate toxicity, as evidenced by elevated seizure severity and duration, hippocampal neuron damage and mortality, as well as altered glial fibrillary acidic protein immunoreactivity and increased microglial infiltration, supporting the relevance of NRF2-ARE signaling in kainate-induced excitotoxicity [340]. Accordingly, the expression of NRF2, NQO1 and HO-1 was slightly but significantly upregulated in the hippocampus after intracerebroventricular injection of kainate. Their expression levels were significantly increased after gintonin treatment, indicating that observed protective activity of gintonin on kainate-induced seizure and hippocampal cell death might be closely associated with its antioxidant activity [341]. In contrast, lower expression of NRF2, SLC7A11, and GPX4 in hippocampal tissue of mice with kainate-induced seizures has been reported. However, pretreatment with quercetin alleviated seizure-like behavior, learning and memory deficits and reversed alterations in NRF2, SLC7A11, and GPX4 levels, suggesting inhibitory effects on NRF2-mediated ferroptosis [339]. These discrepancies could be explained by the differences in the dosage, treatment long and administration route. Indeed, kainate modulate regulation of AKT/GSK-3β axis and NRF2 subcellular localization in a time-dependent manner [342].

Other drug-induced seizure models have been employed to study the role of NRF2, including pilocarpine. After pilocarpine injection, the expression levels of *Nfe2l2* and *Hmox1*, *Nqo1* and *mGst* mRNA increased progressively in mice following status epilepticus, but after 72h declined. Injection of AAV2-hNRF2 in mice resulted in seizure reduction, decreased microglial activation and prevention of hippocampal neuron loss [338]. Using a phenytoin (PHT)-resistant mouse model, antioxidative pretreatment with N-acetylcysteine was shown to prevent and reverse PHT resistance, whereas activation of NRF2 was able to induce PHT resistance, suggesting antioxidative therapy as promising strategy for treatment of drug-resistant epilepsy [343]. Worsened epileptic seizures and cognitive impairment caused by amygdala kindling and hindered response to SFN were observed in NRF2-KO mice [344]. Moreover, prolonged treatment with SFN elevated seizure threshold in the 6 Hz seizure stimulation and fluoroethyl-induced seizure model, as well as protected mice against pilocarpine-induced status epilepticus. In contrast to findings suggesting SFN anticonvulsant activity due to antioxidant effects and enhanced mitochondrial function, SFN injected acutely at a toxic dose exerted proconvulsant activity in both chemically and electrically induced seizures in mice [345].

4.4.8. Mouse models of neurodegenerative diseases

The involvement of NRF2 in the development of neurodegenerative diseases has been demonstrated in a multitude of studies (Table 10) [346–351]. Neurodegenerative diseases are characterized by oxidative stress, mitochondrial alterations, loss of proteostasis leading to the appearance of protein aggregates, and neuroinflammation, with aging being a major risk factor. The aging process is the main cause of the decline in NRF2 activity, which interconnects NRF2 with neurodegenerative diseases. To date, it has not been described that defects in NRF2 are the cause of the neurodegenerative process, but it has been described that several polymorphisms in NRF2 are associated with a more exacerbated progression of these diseases or, on the contrary, may be protective [352–356]. All this evidence points to the transcription factor NRF2 as a key player in the molecular events underlying neurodegeneration and thus many studies have been conducted to unravel the role of NRF2 in these diseases. The use of NRF2-deficient mice in conjunction with models of the different neurodegenerative diseases has led us to understand the role of NRF2 in the neurodegenerative process and its potential use as a therapeutic target to develop new therapies.

In relation to AD the most prevalent neurodegenerative disorder worldwide, it has been described that the absence of NRF2 in different transgenic mouse models exacerbates memory loss and cognitive impairment in general, due to an increase in the accumulation of Aβ plaques and TAU phosphorylation, the two main molecular characteristics of this disease [357–360]. Induction of NRF2 (either by gene overexpression or pharmacological modulation) has therapeutic potential that has been evidenced in different transgenic models recapitulating several AD hallmarks (Table 10) [361–365]. Based on these evidence, DMF, a potent NRF2 inducer, has been proposed for clinical trials for AD [366].

The relevance of the role of NRF2 in Parkinson's disease (PD) has also been studied in detail with the use of toxin-based models (6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone) or protein overexpression models, such as overexpression α-synuclein, main component of Lewy bodies. In all these models, the absence of NRF2 exacerbates clinical features of the disease, such as loss of dopaminergic neurons, increased reactive gliosis, exacerbated oxidative stress or mitochondrial alterations [367–371] (Table 10). As in AD models, the therapeutic potential of NRF2 activation has been studied in different PD models. In the models analyzed, the different NRF2 inducers had beneficial effects on dopaminergic neurodegeneration, neuroinflammation and oxidative stress [372–376].

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) present an overlap at clinical, pathological, and genetic levels, and represent two ends of a spectrum disorder [377]. The first mutation found to be related to the development of ALS was in the gene coding for SOD1 [378], and therefore, most of the studies that have been performed to date in relation to ALS and NRF2 are in SOD1 transgenic mice (Table 10). The data provided by these studies indicate a role of NRF2 preferentially at the astrocyte level, since NRF2 deficiency at the neuronal level or in skeletal muscle fibers has little impact on disease progression [379–381]. In this model, the effectiveness of different NRF2-inducing compounds has been analyzed [382–384], and Edaravone has recently been approved for clinical use in ALS patients [385]. On the other side, although AD is the most frequent dementia, between 60 and 70 % of cases, FTD is the second cause of dementia in adult patients and the most frequent in patients under 65 years of age [386]. Based on the major protein component, a classification of the disease can be established into two predominant histopathological subgroups, both present in approximately 45 % of patients with FTD [1]: FTD-TAU associated with mutations in the gene that codes for TAU (*MAPT*) and [2] FTD-TDP-43. The relevance of NRF2 in FTD development has been analyzed in several murine models recapitulating various features of the disease. In all models, pharmacological activation of NRF2 can significantly reverse the neurodegenerative process [387–390].

In other neurodegenerative diseases of lower prevalence in the population, such as Huntington's disease or Friedreich's ataxia, the use of NRF2 activators was also capable of reversing the neurodegenerative process (Table 10) [391–394]. All this evidence, obtained by combining NRF2-deficient mice with models of the various neurodegenerative diseases, has demonstrated the benefit of pharmacological modulation of NRF2 as a therapeutic strategy applicable to patients with these diseases. This has led to the recent approval of omaveloxolone, an NRF2 inducer, as a treatment for Friedreich's ataxia [395].

4.4.9. Mouse models of retinal diseases

Age-related macular degeneration (AMD) is the most common cause of blindness in the Western world. It has no definitive treatment and can lead to vision loss and decreased quality of life in older adults [396]. The primary cause of pathology appears to be the retinal pigment epithelium (RPE) degeneration, together with the accumulation of extracellular deposits called drusen, between the RPE and the choroid. The heavy, daily, phagocytic load on the RPE, together with a highly oxidative environment and high metabolic activity, leads to the slow accumulation of indigestible material over decades. Consistently, a hallmark of AMD is the accumulation of lipofuscin in the RPE. Lipofuscin is thought to be a by-product of photoreceptor outer segments (POS) phagocytosis and results from incomplete digestion due to lysosomal dysfunction [397,398]. Lipofuscin occurs naturally and accumulates gradually with ageing, but it is exacerbated in AMD, being a predictive marker of retinal stress, evaluated by fundus autofluorescence of the eye [399,400]. Lysosomal dysfunction in RPE is postulated to be at the center of a pathogenic hub contributing to proteotoxicity, mitochondrial dysfunction, redox imbalance, and inflammation in AMD [401,402], eventually leading to RPE cell death and vision loss. Thus, it is likely that dysfunction within lysosomes can cause dysfunction in other organelles, leading to cell death. Also, photodamage of lipofuscin may induce oxidative stress further contributing to AMD. In fact, the retina is especially prone to oxidative stress due to high oxygen pressure and exposure to UV and blue light, promoting ROS generation [403]. Altogether, current evidence suggests a key role of the antioxidant response, autophagy, and lysosomal function in the maintenance of a healthy RPE.

NRF2, as the master regulator of the antioxidant response, has been implicated in human AMD and prospected as a therapeutic target (Table 11). It has been shown that the activity of NRF2 in the RPE of aged mice has been found to be impaired, indicating that the aging RPE has a limited capacity to respond to perturbations in ROS levels, and suggesting that an impaired antioxidant response by aging RPE after an oxidative stimulus could induce RPE dysfunction that may contribute to the development of AMD [404].

Thus, the NRF2-KO mice can provide a novel model for mechanistic and translational research on AMD. These mice showed RPE damage, namely drusen-like deposits, increased autofluorescence and sub-RPE deposition of inflammatory proteins, resembling dry AMD. Also, the RPE accumulates large amounts of autophagosomes and autolysosomes, due to impaired macroautophagy, suggesting that deregulated autophagy is likely a mechanistic link between oxidative injury and inflammation [405]. Importantly, these phenotypes are exacerbated by exposure of NRF2-KO mice to mild white light [406]. Also, a single-nucleotide polymorphism in the NRF2 encoding gene was associated with the risk for AMD [407].

While there is some data supporting a beneficial effect on NRF2 against AMD, a translational approach using preclinical models is lacking. Evidence suggests that protecting the retina from oxidative stress-induced injury is crucial for managing dry AMD. A recent study showed that overexpression of NRF2 in the RPE with an AAV construct produces a robust rescue effect in a mouse model of retinitis pigmentosa. NRF2 overexpression preserved RPE morphology and upregulated multiple oxidative defense pathways. These are the same pathways that are implicated in the pathogenesis of AMD, suggesting that NRF2 overexpression may be a potential therapeutic strategy to slow the pathogenesis of this disease [408].

Table 10
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in neurodegenerative diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null expression models for NRF2 (pink), models employing NRF2 overexpression (green), and those in which NRF2 has been stimulated employing direct strategies using classical activators (blue) or indirect methods (grey). *Dimethyl fumarate (DMF); intraperitoneal injection (i.p.); intracerebral injection (i.c.); oral administration (p.o.); sulforaphane (SFN); subcutaneous administration (s.c.); 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); 3-nitropropionic acid (3-NP); 6-hydroxidopamine (6-OHDA)*.

Study object	Model	Treatment	Results	PMID
Alzheimer's disease	APP/PS1:NRF2-KO vs APP/PS1: NRF2-WT	None	Both spatial learning and memory abilities are decreased in NRF2-deficient mice. Likewise, in the hippocampus, the accumulation of Aβ and $pTAU^{S404}$ increases, and neuroinflammation is exacerbated, accompanied by an increase in pro-inflammatory cytokines in NRF2-deficient mice. (11–12 months old-male and female). Also, increase in Aβ and interferon-gamma (IFNγ) levels, and microgliosis. Deficiency in NRF2 leads to increased intracellular levels of APP, Aβ [1–42], and Aβ [1–40], without a change in total full-length APP. Increased APP/PS1-mediated autophagic dysfunction together with accumulation of multivesicular bodies, endosomes, and lysosomes.	32454936 [357], 29036636 [358], 25316599 [359]
	APP/TAU::NRF2-KO vs APP/TAU::NRF2-WT	None	These mice combined amyloidopathy and tauopathy together with NRF2-deficiency, and showed deficits in spatial learning, memory and reduced long term potentiation in the perforant pathway. Furthermore, they presented increased markers of oxidative stress and neuroinflammation as well as higher levels of insoluble phosphorylated-TAU and Aβ*56.	28704727 [360]
	APP/PS1	LV-hNRF2 or LV-GFP (i.c. 2 μl of 1.71×10^9 in dentate gyrus)	Lentiviral vector delivery of NRF2 into the hippocampus of 9-month-old transgenic AD mice (APP/PS1 mice) reduced spatial learning deficits, decreased astrocytic but not microglial activation.	19805328 [361]
	$APP/PS1:NRF2^{GFAP}$ vs APP/PS1: NRF2-WT (astrocytic NRF2 target gene induction)	None	They demonstrate astrocytic NRF2 target gene induction in AD (homozygous at 12- or 20-weeks female mice were used for all experiments). Astrocytic NRF2 activation promotes glutathione production and release into the extracellular space.	35013236 [491]
	APP^{NLGF}–Keap1FA/FA	None	Genetic NRF2 activation by Keap1 gene knockdown led to the induction of glutathione synthesis and the repression of inflammatory cytokine gene expression and is able to suppress AD-like pathology in model mice.	31932477 [492]
	APP/PS1	Fasudil (i.p. 25 mg/kg/day) for 2 months	Fasudil treatment improved learning and memory ability, elevated the concentration of antioxidative substances and decreased lipid peroxides, as well as inhibited neuronal apoptosis.	33969676 [493]
	APP/PS1	SFN (p.o. gavage 25 mg/kg/day) for 5 months	SFN treatment ameliorated behavior, cognitive impairment and decreased Aβ plaques.	28507518 [363]
	APP/PS1	POZL (inhibitor KEAP1-KNR2 interaction) (p. o. gavage 40 mg/kg/day) for 60 days	POZL treatment improved learning and memory dysfunction, reduced hyperphosphorylation of TAU, and the synaptic function was recovered.	37385075 [494]
	APP/PS1	DMF (p.o. gavage 75 mg/kg) for 40 days	They did not see improvement in water maze performance, β-amyloid accumulation, plaque formation, microglia activation.	34233173 [364]
	APP/PS1	Resveratrol (chow 1 %; 16 mg/kg/day)	Resveratrol treatment significantly prevented memory loss, reduced the amyloid burden and increased IL1β and TNF gene expression	25024312 [495]
	3 × Tg-AD mice	SFN (i.p. 5 or 10 mg/kg SFN every other day) for 2 months. SFN (p.o. gavage 50 mg/kg/day 6 days a week) for 8 weeks	Activation of NRF2 by treatment with SFN suppressed BACE1 and BACE1-AS expression and Aβ production and ameliorated cognitive deficits and AD-related pathologies in 3xTg-AD mice. SFN treatment do not affect mRNA expression of amyloid precursor protein or TAU, however increased increase levels of CHIP and HSP70.	31164420 [496], 29714053 [497]
	5 × FAD	SFN (i.p. 5 or 10 mg/kg every other day) for 2 months	5xFAD-NRF2-KO mice exhibited significantly elevated Bace1 and Bace1-AS expression, increased Aβ plaque pathology, and more severe cognitive impairment. Activation of NRF2 by treatment with SFN suppressed	31164420 [496]

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Table 10 (*continued*)

Study object	Model	Treatment	Results	PMID
			BACE1 and BACE1-AS expression and Aβ production and ameliorated cognitive deficits and AD-related pathologies in 5xFAD.	
	AD-like mice induced by Aβ1–40	SFN	SFN ameliorated cognitive function of Aβ-induced AD mouse model however, SFN did not involve inhibition of Aβ aggregation.	23253046 [498]
	APP/TAU	DMF (p.o. 100 mg/kg/day once every two days) for six weeks	DMF, protected from disease progression in this animal model.	30029164 [365]
	Tg^{19959}	CDDO-MA (p.o. 800 mg/kg of chow) for 3 months	CDDO-MA treatment significantly improved spatial memory alterations and reduced plaque formation, microgliosis, and oxidative stress in the transgenic mice.	19200343 [390]
	$PS1^{V97L}$-Tg	SFN (i.p. 5 mg/kg/day) for 120 days	SFN treatment significantly inhibited oxidative stress, reduced Aβ accumulation, and reversed the cognitive function decline in this mouse model. SFN treatment prevented form cognitive decline.	30461032 [499], 29614663 [500]
Parkinson's disease	NRF2-KO vs NRF2-WT	6-OHDA (i.c. 4 μg in 1 μl into the right striatum) and transplantation of astrocytes overexpressing NRF2	NRF2 knockout mice displayed increased vulnerability to 6-OHDA. Animals transplanted with adeno-NRF2 infected astrocytes demonstrated significantly reduced lesion with 6-OHDA	17336276 [368]
	NRF2-KO vs NRF2-WT	MPTP (i.p. 20 mg/Kg/day) for 4 weeks	NRF2-deficient mice showed exacerbated gliosis and dopaminergic nigrostriatal degeneration	20676377 [369]
	NRF2-KO vs NRF2-WT	MPTP (i.p. 20 mg/Kg/day) for 4 weeks	Basal ganglia of NRF2-KO mice exhibited a more severe dopaminergic dysfunction than wild type littermates in response to MPTP, more astrogliosis and microgliosis. Inflammation markers characteristic of classical microglial activation was also increased and, at the same time, anti-inflammatory markers attributable to alternative microglial activation were decreased.	19908287 [501]
	NRF2-KO vs NRF2-WT	3H-1,2-dithiole-3-thione (p.o. gavage 67 mg/kg/day) was given once 2 days at day 1, 3 or 5 before MPTP (i.p. 40 mg/Kg divided into four injections at 2h intervals)	D3T treatment led to partial protection against MPTP-induced neurotoxicity. NRF2-KO animals showed no protection from this dose of MPTP at any level of the striatum	16959318 [502]
	NRF2-KO vs NRF2-WT	MPTP (i.p. 20 mg/Kg/day). SFN (i.p. 50 mg/kg/day every other day) for 6 days	SFN partially rescued MPTP-induced loss of dopaminergic neuron and attenuated astrogliosis and microgliosis only in wild type mice.	21254817 [503]
	NRF2-KO vs NRF2-WT	DMF (p.o. gavage 10, 50, or 100 mg/kg twice a day 12 h apart at a cumulative dose). 1 d before MPTP and daily for 5 d after MPTP. MPTP (i.p. 10 mg/kg three times a day 2 h apart) for 5 days	DMF exerted neuroprotective effects and blocked MPTP neurotoxicity in wild type but not in NRF2 null mice	27277809 [374]
	NRF2-KO vs NRF2-WT	AAV6-α-SYN or AVV6-GFP (i.c. 2 μl of 4.0×10^{12} injected at substantia nigra)	AAV-α-synuclein injection in NRF2-deficient mice exhibited exacerbated degeneration of nigral dopaminergic neurons and increased dystrophic dendrites, which correlated with impaired proteasome gene expression and activity. NRF2 lack also exacerbated neuroinflammation.	22513881 [367]
	NRF2-KO vs NRF2-WT	Ellagic acid (20 or 100 mg/kg/day) beginning 30 min before rotenone (s.c. 1 mg/Kg/day 6 times per week) for 5 weeks	Ellagic acid activated NRF2 pathway in rotenone-induced dopaminergic neuronal loss and had neuroprotective and anti-neuroinflammatory effects. These beneficial effects have been damped in NRF2-null mice.	32657027 [370]
	NRF2-KO vs NRF2-WT	AAV-6-α-SYN (i.c. 2 μl of 1.7×10^8 injected at substantia nigra). DMF (p.o. gavage 100 mg/kg/day) every day for 1 and 3 weeks and every two days for 8 weeks.	DMF protected nigral dopaminergic neurons against α-SYN toxicity and decreased astrocytosis and microgliosis.	27009601 [504]
	humanized $\alpha\text{-Syn}^+$/NRF2-KO vs humanized $\alpha\text{-Syn}^+$/NRF2-WT	None	h-$\alpha\text{-synuclein}^+$/NRF2-KO mice exhibited increased phosphorylation and oligomerization of α-synuclein together with a loss of tyrosine hydroxylase expressing dopaminergic neurons in the substantia nigra, and more pronounced behavioral deficits.	34221542 [371]
	Wild type	6-OHDA (i.c. 4 μg in 1 μl into the right striatum). DMF (p.o. gavage 50 mg/kg/day) for 7 days prior to lesioning	DMF treatment ameliorated dopaminergic neurodegeneration.	25449120 [505]

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Table 10 (*continued*)

Study object	Model	Treatment	Results	PMID
	Wild type	6-OHDA (i.c. 4 μg in 1 μl into the right striatum). SFN (i.p. 5 mg/kg twice a week) for four weeks after lesion	SFN ameliorated behavior impairments in this PD model and has anti-apoptotic effects in the SN.	23518299 [372]
	Wild type	DMF (p.o. gavage 10, 30 and 100 mg/kg) starting 24 h after the first MPTP injection and continuing through 7 additional days after the final MPTP dosing. MPTP (i.p. 10 mg/kg three times a day 2 h apart) for 7 days	DMF prevented dopamine depletion, increased tyrosine hydroxylase and dopamine transporter activities, and reduced the number of α-synuclein-positive neurons	28006954 [506]
	Wild type	CDDO-EA, CDDO-TFEA 4, 2, 1, and 0.5 μmol of the respective drugs once a day for 4 days before MPTP and once a day for 3 days after MPTP. MPTP (30 mg/Kg/day) for 5 days	Both compounds reduced MPTP-induced oxidative stress and inflammation, and ameliorated dopaminergic neurotoxicity in mice	22746536 [507]
	Wild type	CDDO-MA-containing diet for one week before MPTP injection and 7 days after the MPTP injection (10 mg/Kg for 3 times with an interval of 2 h) for 7 days	CDDO-MA led to significant protection against MPTP-induced nigrostriatal dopaminergic neurodegeneration, α-synuclein accumulation and oxidative damage in mice	19484125 [508]
	Wild type	Rotenone (i.p. 1.5 mg/kg/day) and DMF (p.o.15, 30 or 60 mg/kg/day) for 21 days	Pre-treatment with DMF reduced dopaminergic neuronal loss by promoting autophagosome formation and inhibiting apoptosis	37315723 [375]
	Wild type	Rotenone (i.p. 2.5 mg/kg/day) and Curcumin pre-administration (p.o. 80 mg/kg/day) for 7 days or co-treatment with rotenone for 35 days	Curcumin treatment restored the motor coordination and anti-oxidative activity due to improving the mitochondrial function. Curcumin enhanced autophagy-mediated clearance of misfolded α-synuclein protein	36989171 [509]
	Wild type	Rotenone (p.o. 30 mg/kg/day) and SFN (i.p. 50 mg/kg every other day) for 60 days	SFN treatment inhibited the rotenone-induced reactive oxygen species production, neuronal apoptosis, and rescued rotenone-inhibited autophagy.	27553905 [510]
Amyotrophic lateral sclerosis and frontotemporal dementia	$SOD1^{G93A}$::NRF2-KO vs $SOD1^{G93A}$::caNRF2$^{(\Delta Neh2)}$ *(neuronal expression of a KEAP1 insensitive NRF2 mutant)* vs $SOD1^{G93A}$::MLC-NRF2$^{(\Delta Neh2)}$ *(muscle expression of a KEAP1 insensitive NRF2 mutant) vs* $SOD1^{G93A}$::NRF2-WT	None	Not observe any detrimental effect associated with the lack of NRF2 or its restricted overexpression in neurons or type II skeletal muscle fibers. Modest impact on the course of disease by deleting NRF2 gene.	23418589 [380] 23711824 [381]
	TAU^{P301S}::NRF2-KO vs TAU^{P301S}::NRF2-WT	None	NRF2 deficiency accelerated the onset of hind-limb paralysis and impaired cognitive decline without affecting markers of senescence.	36976489 [511]
	NRF2-KO vs NRF2-WT	AAV1/2-TAU^{P301L} (i.c. 2 μl of 10^8 at hippocampus)	NRF2-KO mice exhibited increased microgliosis and astrogliosis in response to neuronal TAU^{P301L} expression	24277722 [512]
	$SOD1^{H46R/H48Q}$::$NRF2^{GFAP}$ vs $SOD1^{H46R/H48Q}$ $SOD1^{G93A}$::$NRF2^{GFAP}$ vs $SOD1^{G93A}$ *(Astrocytic NRF2 over-expression)*	None	Over-expression of NRF2 in astrocytes significantly delayed onset and extended survival.	19074031 [379]
	$SOD1^{G93A}$	LV-GFP, one of the three genes or a mixture of lentiviruses carrying EAAT2, GDH2, and NRF2. Injected both intra-cisternal and intra-muscularly (5×10^8)	Novel multifactorial-cocktail treatment, using lentiviruses encoding: EAAT2, GDH2, and NRF2, that act synergistically, leading to improvement in body-weight loss, reflex score, neurologic score, and motor performance.	26691332 [513]
	Wild type	AAV2/9-TAU^{P301S}-EGFP; AAV2/9-hNRF2-mCherry (i.c. 1 μl of 5×10^{12} vg/m) or AAV2/9-sgNRF2-Cas9 (i.c. 1 μl of 1×10^{13} vg/ml) at CA3	Overexpressing NRF2 ameliorated TAU^{P301S}-reduced synaptic proteins and synapse.	35917404
	$SOD1^{G93A}$	CDDO-TFEA (p.o. 400 mg of/kg of food, 80 mg/kg body weight/day) for 110 days	The treatment attenuated weight loss, enhanced motor performance, and extended the survival of $SOD1^{G93A}$ mice	21457778 [514]
	$SOD1^{G93A}$	RTA-408 (i.p. 1 mg/kg/day twice weekly) for 6 weeks	ALS mice treated with RTA-408 showed improvement in body weight and motor function.	37352984 [384]
	$SOD1^{G93A}$	Edaravone (i.p. 15 mg/kg/day) for 28 days	Edaravone alleviates the degeneration of both motor neurons and muscles related to oxidative stress.	30565312 [515]
	$SOD1^{G93A}$	Honokiol (i.p 10, 30 or 90 μg/kg/day) or edaravone (i.p. 15 mg/kg/day) from the disease onset to end-stage of disease	Honokiol extended the lifespan of the SOD1-G93A transgenic mice and improved the motor function.	36873166 [383]
	$SOD1^{G93A}$	S [+]-Apomorphine (s.c. 5 mg/kg/day) from 21 days of age through to end-stage	The treatment led to NRF2 induction, and significant attenuation of motor dysfunction in the ALS mice.	23608463 [382]
	$SOD1^{G93A}$	Tetramethylpyrazine nitrone (i.p 10, 30 or 60 mg/kg/day) beginning at disease onset for four weeks	TBN treatment slowed the progression of motor neuron disease due to improved motor performance, reduced spinal motor neuron loss	33152451 [516]

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Table 10 (*continued*)

Study object	Model	Treatment	Results	PMID
			and the associated glial response, and decreased skeletal muscle fiber denervation and fibrosis.	
	Wild type	AAV1/2-TAU^{P301L} (i.c. 2 μl of 10^8 at hippocampus). SFN (i.p. 50 mg/kg/day) for 21 days	SFN treatment was able to modulate astrogliosis but failed to reduce microgliosis induced by TAU^{P301L} in Cx3cr1$^{-/-}$ mice.	30769286 [389]
	Wild type	AAV1/2-TAU^{P301L} (i.c. 2 μl of 108 at hippocampus). DMF (p.o. gavage 100 mg/kg/day) for 21 days	DMF modulates TAU phosphorylation, neuronal impairment, and neuroinflammation produced by TAU^{P301L} overexpression.	29121589 [388]
	Wild type	AAV9-TDP-43^{M337V} ($2.5x10^{10}$ vg at striatum bilaterally). Starting at second day after the injection Tetramethylpyrazine nitrone (p.o. 30 mg/Kg twice per day)	TBN treatment led to improved motor function and prolonged survival rate.	33929499 [387]
	TAU^{P301S}-Tg	Methylene blue (p.o. 4 mg/kg or 40 mg/kg of chow)	MB treatment improved the behavior abnormalities and reduced TAU pathology, inflammation and oxidative damage.	24556215 [517]
	TAU^{P301S}-Tg	Benfotiamine (p.o. 200 mg/kg/day in diet)	Benfotiamine administration significantly ameliorated mitochondrial dysfunction and attenuated oxidative damage and inflammation.	29860433 [518]
	SOD1:ARE-hPAP	None	This model showed that NRF2 activation appears to progress in a retrograde way along the motor pathway.	17631292 [519]
Huntington's disease	NRF2-WT, NRF2-KO and ARE-hPAP and NRF2-overexpressing astrocytes	3-NP (i.p. 50 mg/kg every 12 h) for a total of seven injections. Malonate (i.c. 1 μl, 0.5 M unilaterally at striatum)	NRF2-deficient mice are significantly more vulnerable to 3NP or malonate. Transplantation of NRF2-overexpressing astrocytes before lesioning conferred protection against 3-NP	15611470 [520]
	R6/2 and YAC128	DMF (p.o. 30 mg/kg twice daily)	DMF prevented from weight loss and attenuated motor impairment, consequence of preservation of neurons in the striatum as well as in the motor cortex.	21297955 [391]
	N171-82Q	CDDO-EA (p.o. 100 mg/kg or 200 mg/kg diet); CDDO-TFEA 1 (p.o. 100 mg/kg, 200 mg/kg or 100 mg/Kg diet)	The treatment with CDDO-EA or CDDO-TFEA lead to reduced oxidative stress, improved motor impairment and increased longevity together with rescued striatal atrophy in the brain.	20338236 [392]
	Wild type	SFN (i.p. 5.0 mg/kg/day) from 30 min before first 3-NP treatment (i.p. twice daily for 2 days at 12h-intervals at a dose of 60 mg/kg on the first day and 80 mg/kg on the second day)	Pre-treatment with SFN was able to modulate formation of a lesion area, neuronal death, succinate dehydrogenase activity, apoptosis, microglial activation.	26096705 [393]
	Wild type	Gintonin (p.o. 25, 50 and 100 mg/kg/day). 3-NP (i.p. twice daily for 2 days at 12h-intervals at a dose of 60 mg/kg on the first day and 80 mg/kg on the second day)	GT has beneficial effects with a wide therapeutic time-window in 3-NP model of HD due to modulation of antioxidant and anti-inflammatory defenses.	30853569 [521]
	Wild type	3-NP (i.p. twice daily for 2 days at 12h-intervals at a dose of 60 mg/kg on the first day and 80 mg/kg on the second day). *Schisandra chinensis* (p.o. 75, 150, or 300 mg/kg)	The gomisin A and schizandrin components of *Schisandra chinensis* significantly reduced the neurological impairment and lethality induced by 3-NPA treatment via NRF2 and MAPK activation	29033839 [522]
Spinal and bulbar muscular atrophy	AR97Q	ASC-JM17 (p.o. 120 mg/kg/day) for 16 weeks	ASC-JM17 ameliorates toxicity of the mutant androgen receptor in a mouse models.	26962150 [523]
Friedreich Ataxia	YG8R	Dyclonine (i.p. 1–10 mg/kg/day) for 1 week	The treatment with dyclonine prevented a performance decline in balance beam studies in a FA mouse model.	25113747 [394]

Although there are no current FDA-approved drugs for AMD, an early clinical study called Age-Related Eye Disease Study (AREDS) showed that taking a high-dose supplementation with antioxidant compounds such as vitamins C and E and β-carotene can help prevent the progression of AMD and reduce the risk of advanced AMD progression by 25 % and moderate vision loss by 19 % within 5 years [409]. Newer formulations (AREDS2) replace the β-carotene in the original AREDS formula, with lutein/zeaxanthin. This new formula (AREDS2) is now used as the first-line treatment for dry AMD. Flavonoids, such as quercetin, have free radical-scavenging and antioxidant functions, and can activate NRF2. Correspondingly, preparation of a solid dispersion that significantly improved the solubility and dissolution rate of quercetin, thereby increasing its bioactivity, showed a protective effect on retina oxidative injury in a mice model of AMD, which was associated with activation of NRF2 signaling and related antioxidant enzymes [410].

In summary, there is mounting clinical and experimental evidence supporting chronic oxidative stress as a driving force in AMD pathology (Table 11). Therefore, understanding the pathways counteracting oxidative stress in the RPE, especially the NRF2 pathway, in the context of aging, will provide new therapeutic strategies for AMD.

4.4.10. Mouse models of cancer

In contrast to the acute physiological regulation of NRF2, in neoplasia there is evidence for increased basal activation of NRF2. Indeed, somatic mutations that disrupt the KEAP1-NRF2 interaction have been identified, indicating that NRF2 activation may facilitate tumor progression. The fact that expression of K-Ras^{G12D} reduced ROS levels and increased NRF2 protein levels in a KEAP1-independent manner paves the way to the analysis of NRF2 activation in a tumorigenic context employing mouse models. It is not possible to discuss in

Table 11
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in retinal diseases. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are null expression models for NRF2 (pink), models employing NRF2 overexpression (green).

Study object	Model	Treatment	Results	PMID
Age-related macular degeneration	NRF2-KO vs NRF2-WT	None	RPE damage, namely drusen-like deposits, increased autofluorescence and sub-RPE deposition of inflammatory proteins. RPE accumulates large amounts of autophagosomes and autolysosomes, due to impaired macroautophagy	21559389 (405)
	NRF2-KO vs NRF2-WT	1 % tropicamide under dim red light and 4500 ± 300 or 10000 ± 2000 lux cold fluorescence illumination for 2 h or 3 h. 10 μL GCCNP (intravitreally 1 mg/ml) 3 days after light exposure	This animal model developed phenotypes of photoreceptor degeneration, retinal function impairment, ROS accumulation, and inflammation reaction in a relatively shorter. The delivery of GCCNP protected retina against progressive retinal oxidative damage.	31601909 (406)
	NRF2-KO vs NRF2-WT	Mice were fed normal diet during months 1–3, then high-fat diet, received the intake of hydroquinone dissolved in the drinking water (0.8 %) during months 4–6, and were intragastric administrated with Q-SD, 200 mg/Kg, (suspended in 0.5 % CMC-Na) daily during months 7–9	Q-SD exhibited more potent protective effects on retina oxidative injury in model mice of dry AMD, which were associated with activation of NRF2 signaling and related antioxidant enzymes	31781321 (410)
Retinitis pigmentosa	*Pde6b*^{rd10}	AAV8-hRedO-NRF2 (subretinal injection of 0.3 μl of 3 × 10^8 per eye)	NRF2 overexpression preserved RPE morphology and upregulated multiple oxidative defense pathways	33491671 (408)

detail the numerous mouse models of cancer, where the role of NRF2 has been investigated; here, we just show a small number of examples (Table 12). Thus, NRF2-deficient pancreata were noted to contain fewer PanIN. While the proliferation of the salivary gland was unchanged, PanIN from NRF2-deficient mice were significantly less proliferative and demonstrated an increased content of cells exhibiting senescence-associated β-galactosidase activity. The role of NRF2 in lung cancer development was investigated. NRF2 deficiency resulted in a significant reduction in lung cancer burden, and an increase in median survival [411].

There are multiple factors that determine the control interpreting the role of NRF2 in cancer. Several studies were suggesting that NRF2 suppression could enhance cancer prognosis. For example, in an interesting study, the researchers injected BALB/c nude mouse models with CaoV3 cells to study ovarian cancer. Then, they treated the mice with eriodictyol, showing a significant reduction in NRF2 expression, which led to inhibition of tumor growth [412]. In line, recent study evidence that NEDD4 Like E3 Ubiquitin Protein Ligase (NEDD4) inhibits migration, invasion, cisplatin resistance and promotes apoptosis of bladder cancer cells by inactivating the p62-KEAP1-NRF2 pathway [413]. The combined administration of vitamin C and erastin modulated the AMPK/NRF2/HMOX1 pathway, enhancing ferroptosis in pancreatic cancer cells in mice injected with Panc02 cells [414]. In the case of liver cancer, a study with nude mice injected with SMMC-7721 cells to induce liver cancer explored the impact of overexpression of NRF2. The results indicated that tumors from SMMC-7721-NRF2 cells exhibited significantly larger size compared to those from SMMC-7721-Vector cells [415]. These findings were confirmed in melanoma mouse models, where NRF2-KO melanoma cells were used to induce melanoma. Notably, 40 % of mice injected with NRF2-KO cells showed no tumor growth, in contrast to all mice injected with control melanoma cells [416]. These findings were also confirmed in mouse models of lung cancer. For example, BALB/c nude mice were injected with A549 cells to induce lung metastasis. The research showed a decrease in NRF2 protein levels in mice treated with erastin compared to the control group [417]. In another study, C57BL/6 mice were injected with B16–F10-Luc cells to study the effects of pedunculoside, a plant triterpene saponin. Pedunculoside significantly reduced lung metastasis by reversing the expression of EMT proteins, lowering ROS production, and suppressing the MAPK and NRF2 pathways [418]. Moreover, Nu/nu nude mice inoculated with A549 tumor cells were used in the study. Luteolin treatment aimed to suppress the NRF2 pathway *in vivo* [419]. Paradoxically, in the case of colorectal cancer (CRC), the nature of the xenograft or the pharmacological agent used to induce CRC seems to play a critical role in exploring the role of NRF2. For example, nude mice injected with HT-29 cells with/without NRF2-KO were used to explore the impact of Ibrutinib. The study found that Ibrutinib enhanced the sensitivity of CRC cells to ferroptosis inducers by suppressing NRF2 [420]. Additionally, it was reported that NOD/SCID mice injected with SW620-Luc2 cells to induce colorectal carcinoma found that curcumin prevented colorectal cancer metastasis by promoting miR-34a, initiating a ROS/KEAP1/NRF2/miR-34a/b/c signaling cascade [421]. Conversely, NRF2-KO mice were more susceptible to dextran sodium sulfate (DSS)-induced colitis [422], a major risk factor for the development of CRC. In mice with NRF2-KO, a model with 2.5 % (w/v) DSS in drinking water + AOM (10 mg/kg) with/without 17β-estradiol (E2) was studied. The research found that 17β-estradiol effectively suppressed the development of colorectal cancer induced by azoxymethane/DSS in male mice lacking NRF2 [423]. Analysis of combined occurrences of preneoplastic and neoplastic lesions in mice lacking NRF2 when administered potassium bromate (KBrO3), to induce intestinal carcinogenesis, revealed notable rise in the number of both lesion type [424]. However, neither genetic nor pharmacological NRF2 activation, nor disruption of NRF2, affected colorectal adenoma formation in a mouse model of familial adenomatous polyposis (FAP) [425], suggesting that NRF2 activation is unlikely to impact the early stages of colorectal cancer development. The disparities could also be noticed on epigenetic levels. For example, using the transgenic adenocarcinoma of mouse prostate (TRAMP) model, an epigenetic study was conducted, revealing that NRF2 expression was epigenetically suppressed [426]. Taken together, these observations suggest that the role of NRF2 in cancer could depend on the type of cancer investigated, the mouse model used, the pharmacological agent used to induce cancer, and the type of study.

4.5. *Rat models (animalia chordata mammalia Rattus norvergicus)*

Although the majority of investigations on NRF2 are performed on mouse models, there are few studies that describe the rat as a model for mechanistic or interventive studies on NRF2 (Table 13). Rats are considered to be more suitable for physiological and toxicological studies than mice because of their larger body size [427] and liver response to some toxins such as aflatoxin B_1 [428,429].

The development of new technologies for genetic modifications by the TALEN methodology, as well as the establishment and culturing of embryonic cells, allowed the generation of the first NRF2-KO rat model (*SD-Nfe212*^{em1Mcwi}) in a Sprague-Dawley background. These rats presented a germ line transmission of a 41-bp deletion within the first exon, eliminating the NRF2 translation start codon avoiding NRF2 protein synthesis [427]. The aim of this study was to evaluate the role of NRF2 as the most important antioxidant and cell protective transcription

Table 12
Examples of studies using *M musculus* as model organism to examine the role of NRF2 in cancer. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are models null for NRF2 expression (pink), those in which NRF2 activity has been reduced by genetic (dark yellow) or pharmacological approaches (light yellow), models employing NRF2 overexpression (green) and studies using pharmacological activation of NRF2 by classical compounds (blue). *intraperitoneal injection (i.p.); oral administration (p.o.); subcutaneous administration (s.c.)*.

Study object	Model	Treatment	Results	PMID
Prostate cancer	TRAMP (Transgenic Adenocarcinoma of Mouse Prostate)	None	Epigenetic mechanisms, including promoter methylation and histone modifications, led to the silencing of NRF2 gene expression.	20062804 [426]
	Nude mice injected with PC3 cells	PC-3-pcDNA3.1 or PC-3-dn-NRF2-V5 stable cell lines (s.c. 3×10^6 into the left flank)	Epigenetic mechanisms, including promoter methylation and histone modifications, led to the silencing of NRF2 gene expression.	20062804 [426]
Ovarian cancer	BALB/c nude mice injected with CaoV3 cells	Eriodictyol (100 mg/Kg)	The IHC analysis demonstrated a significant reduction in NRF2 expression due to eriodictyol, which led to the inhibition of tumor growth.	37020356 [412]
Bladder cancer	BALB/c nude mice injected with T24 cells	NEDD4L vectors	NEDD4L hinders the advancement of Bladder cancer by deactivating the p62-Keap1-NRF2 pathway.	37087754 [413]
Pancreatic cancer	C57BL/6 mice injected with Panc02 cells	Erastin (intertumoral injection 400 μM) and/or vitamin C (i.p. 4 g/Kg every 2 days) for 14 days	The combined administration of vitamin C and erastin depleted intracellular GSH, resulting in an increase in ferrous iron levels. This was achieved by modulating the AMPK/NRF2/HMOX1 pathway, thereby enhancing ferroptosis in PC cells.	35915609 [414]
Liver cancer	Nude injected with SSMC-7721	SSMC-7721 cells with stable overexpression of NRF2 or vector control cells (s.c. 1×10^6 into the left flank)	The findings indicate that tumors derived from SMMC-7721-NRF2 cells exhibited notably greater size compared to those originating from SMMC-7721-Vector cells.	31546024 [415]
Melanoma	C57BL/6 mice injected with #781 cells (*Tyr-Cre*ERT2; $BRAF^{CA}$; $Pten^{fl/fl}$)	UACC-62 control or sg*Nfe2l2* CRISPR/Cas9 silenced cells (s.c. 10.000 cells into the flanks)	While every mouse injected with control melanoma cells developed tumors, with an average onset of 17 days, 40 % of mice injected with NRF2 knockout cells showed no tumor growth at all. NRF2 as key regulator of immune-modulating genes, linking oxidative stress with the induction of cyclooxygenase 2 (COX2) in an ATF4-dependent manner.	32978520 [416]
Lung cancer	NRF2-KO vs NRF2-WT	Vinyl carbamate (i.p 0.32 mg/mouse), one week after CDDO-Me/kg (p.o. 12.5–50 mg/Kg in food) for 16 weeks.	CDDO-Me reprogrammed tumor-educated bone marrow-derived macrophages from a tumor-promoting to a tumor-inhibiting phenotype. CDDO-Me significantly reduced tumor burden and improved pathological grade in wild-type but not in NRF2 knockout mice.	36670978 [524]
	BALB/c nude mice injected with A549 cells	Qingrehuoxue g/Kg)	The levels of NRF2 protein expression decreased in the mice treated with erastin compared to the control group.	36860928 [417]
	C57BL/6 mice injected with B16–F10-Luc cells	Pedunculoside (20, 40, 80 mg/Kg)	Pedunculoside significantly reduced lung metastasis in mice by reversing the expression of EMT proteins, reducing ROS production, and suppressing the MAPK and NRF2 pathways.	37209605 [418]
	nu/nu nude mice inoculated with A549 tumor cells	Luteolin (p.o. g 40 mg/kg thrice per week) for 35 days	Luteolin inhibited the NRF2 pathway in mouse liver without apparent toxicity; significantly inhibited the growth of human NSCLC carcinoma xenografts in nude mice; and the combination of luteolin and cisplatin was more effective in inhibiting tumor cell growth than either luteolin or cisplatin alone.	24747074 [419]
Colorectal cancer	NRF2-KO vs NRF2-WT	Azoxymethane (i.p. 10 mg/Kg), one week after dextran sodium sulfate (p.o. 2.5 % (w/v) in drinking water); 17β-estradiol (i.p. 10 mg/kg/day) for one week	17β-estradiol effectively suppresses the development of colorectal cancer induced by azoxymethane/dextran sulfate sodium in male mice lacking NRF2.	33068552 [423]
	NOD/SCID mice injected with SW620 cells-Luc2	SW620-Luc2 cells treated ex vivo with curcumin (14.49 μM) for 48 h (i.v. 4×10^6 at tail vein)	Curcumin prevents the formation of metastases by promoting miR-34a. Curcumin initiates a ROS/KEAP1/NRF2/miR-34a/b/c signaling cascade to inhibit colorectal cancer metastasis.	37210578 [421]
	Nude mice injected with HT-29 cells	Ibrutinib (0.16 mg/ml)	Ibrutinib enhances the sensitivity of CRC cells to ferroptosis inducers by suppressing NRF2.	36823108 [420]
	BALB/c mice injected with CT26	Propofol (200 mg/kg)	Propofol enhances cancer cell metastasis by suppressing ferroptosis through the NRF2 pathway.	36586453 [525]
	BALB/c nude mice treated with HCT-116 cells	Rapamycin (5 mg/Kg/per two days) and garlic-derived S-allylmercaptocysteine (300 mg/Kg/day)	The combination of rapamycin and S-allylmercaptocysteine derived from garlic induces apoptosis in colon cancer cells and inhibits tumor growth in xenograft nude mice by modulating the autophagy/p62/NRF2 pathway.	28737825 [526]
Intestine cancer	NRF2-KO vs NRF2-WT	KBrO3 (p.o 750 or 1500 ppm in drinking water) for 52 weeks	A notable rise in the combined occurrences of preneoplastic and neoplastic lesions was observed in mice lacking NRF2 when administered a high dose of KBrO3.	26899729 [424]

factor in defense of endothelial dysfunction, oxidant stress, and microvascular rarefaction. They reported that the mRNA levels for *Cat*, *Hmox1*, *Sod1 and 2*, *and Gsr* in livers of NRF2-KO rats were lower than those in wild-type rats, and that NRF2 is necessary for the protective effect of low-dose angiotensin II (AngII) infusion to ameliorate endothelial dysfunction and prevent microvascular rarefaction in the face of an elevated dietary salt intake [430].

The same year a publication by Taguchi et al. (2016) reported the generation of a new NRF2-KO rat model. Two transgenic lines were developed, *F344-Nfe2l2em1Kyo* and *F344-Nfe2l2em2Kyo* (the first line

Table 13
Examples of studies using *R norvergicus* as model organism to examine the role of NRF2 in NCDs. The NCDs under study (study subject column) strains used (model column), experimental conditions used (treatment column), results obtained (results column) and article reference number in PubMed (PMID column) are shown. Highlighted are models null for NRF2 expression (pink) and those in which NRF2 has been stimulated employing direct strategies using classical activators (blue) or indirect methods (grey). *Choline-deficient L-amino acid-deficient diet (CDAA); High fat diet (HFD); intravenous injection (i.v.); metabolic associated fatty liver disease (MAFLD); Metabolic associated steatohepatitis (MASH); oral administration (p.o.); sulforaphane (SFN); subcutaneous administration (s.c.).*

Study	Model	Treatment	Results	Reference (PMID)
Lung injury	NRF2-KO vs NRF2-WT	Exposure to room air (normoxia) or > 95 % O2 (hyperoxia) for 48 h	NRF2-ARE signaling pathway provides some protection against lung injury in hypoxia-induced lung injury in rats	34738958 (435)
Cardiovascular diseases	NRF2-KO vs NRF2-WT	Low dose angiotensin II 100 ng/kg/min	Low-dose ANG II infusion reversed salt-induced endothelial dysfunction in MCA and prevented microvessel rarefaction in wild-type rats fed HS diet, but not in NRF2−/− mutant rats.	26637559 (430)
	Wild type	SFN (500 μg/kg i.v.) previous *in vivo* ischemia/reperfusion (30′I/60′R)	SFN preserved cardiac function, decreased infarct size, oxidative stress and inflammation after IR. SFN-mediated cardioprotection involves transient NRF2 activation and participation of the aryl hydrocarbon receptor (AhR).	31422078 (527)
	Wild type	Carnosoic Acid (50 mg/kg, p.o. gavage) followed by ISO-induced acute myocardial injury (s.c. 150 mg/kg)	Carnosoic Acid pretreatment increased NRF2 expression levels and reduced myocardial injury via anti-inflammatory, antioxidant and antiapoptotic effects.	26820671 (201)
	Wild type	SFN (2.5 mg/kg body weight) 24 h before transplantation	Recipient preconditioning with SFN protects the heart from IR injury after experimental transplantation.	24126483 (528)
Metabolic diseases	Wild type	SFN (20 mg/kg) was given p.o. to HFD-rats through gavage three times per week for 10 weeks.	SFN improves multiple mitochondrial bioactivities, such as mitochondrial membrane potential, ATP, and the electron transfer chain based on PGC-1α pathway. SFN also activates lipolysis by transcriptionally upregulating adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL).	30578708 (529)
Liver diseases	NRF2-KO vs NRF2-WT	Diethylnitrosamine (DENA) 150 mg/kg, followed by choline-devoid methionine-deficient (CMD), After a one-week recovery period, rats were fed choline-supplemented (CS) or a CMD diet for 10 weeks	NRF2 genetic inactivation led to increased liver injury and chronic compensatory hepatocyte regeneration when rats were fed a CMD diet. And NRF2 missense mutations, known to disrupt the Keap1-NRF2 binding, were present in 65.7 % of GSTP-positive foci	29758334 (431)
	NRF2-KO vs NRF2-WT	oleanolic acid, 100 mg/kg p.o.ly every day for 7 days, and rats in the ANIT group and OA treatment groups received ANIT (60 mg/kg, dissolved in olive oil) on day 5.	Hepatoprotective effects of OA on ANIT-induced cholestatic liver damage in rats NRF2-KO	36343550 (438)
	NRF2-KO vs NRF2-WT	60 mg/kg/day (or 450 mg/kg/day) Protandim supplement for 2 weeks in rats	Protandim supplementation caused a significant reduction in mitochondrial ROS levels in basilar arteries of HS-fed rats compared to untreated rats fed HS diet	31132190 (530)
	NRF2-KO vs NRF2-WT	Aflatoxin B1 (p.o. 25 μg/100g of body weight)	Single dose administration of AFB1 increased hepatotoxicity and binding of AFB1-N7-guanine to hepatic DNA in Nrf2 knockout rats compared with wild-type. Nrf2 knockout rats repeatedly treated with AFB1 were prone to lethality.	27071940 (429)
	NRF2-KO vs NRF2-WT	None	Short WIT induces the transcriptional activities of BT, whereas long WIT depresses them, and the effect was blunted by NRF2 deficiency	30691853 (433)
	Wild type	STZ-HFD (p.o. 5 mg/kg/day GLB and/or 25 mg/kg/day DMF) from the 6th to 17th week	Reduced hepatic steatosis, delayed progression of MAFLD, controlled inflammation and improved antioxidant status	36999408 (432)
	Wild type	CDAA (p.o. 60 mg/kg Oltipraz or 20 mg/kg NK252 daily) for 9 weeks	Oltipraz and NK252 have an antifibrotic effect in a rat model of MASH	23592516 (531)
	Wild type	MCD or MCD + silymarin (0.5 % w/w) for 8 weeks	Silymarin reduced the activation of hepatic steleate, evaluated by counting α-smooth muscle actin (SMA)-positive cells and measuring α-SMA mRNA expression in the liver lysates as well as in HSCs isolated from the experimental animals.	22710359 (532)
Age Macular Degeneration	Light-induced AMD rat model	Diet supplemented with 2000 ppm (0.2 %) curcumin for two weeks; After two weeks, the rats were placed individually in clear plastic cages with wire tops and exposed to 1000 lux light (white cool light) for 3 h	Dietary supplementation of curcumin provided both functional and structural protection of photoreceptor cells against acute light-induced damage in Wistar albino rats	19121385 (442)

harbors a 7bp deletion and the second line harbors a 1bp insertion in the *NFE2L2* gene homozygously deleting it). The mRNA levels of *Gsta3*, *Akra2*, and *Akra3* were reduced in null rats compared to wild-type, and the toxic effect aflatoxin B1 in the liver was aggravated in both NRF2-KO lines [429].

Additionally, another study aiming to demonstrate whether NRF2 activation occurs at early steps of rat hepatocarcinogenesis employed male F344 NRF2KO rats, was presented in 2018 [428]. In this study NRF2-KO rat models were compared to wild type rats, after a single dose of diethyl nitrosamine, followed by choline-devoid methionine-deficient (CMD) diet. After a one-week recovery period, rats were fed a diet containing rats were fed choline-supplemented or a choline-devoid methionine-deficient diet for 10- or 17-weeks. Following the marker GSTP, the results indicated that NRF2 activation is involved in early preneoplastic lesions. NRF2 missense mutations, known to disrupt the KEAP1-NRF2 binding, were present in 65.7 % of GSTP-positive foci. In rats fed a CMD diet, genetic inactivation of NRF2 led to increased liver injury and chronic compensatory hepatocyte regeneration [431]. Recently, in a MAFLD experimental rat model, showed the efficacy of multiple drugs including DMF in a diabetic-MAFLD rat model. Treatment with DMF significantly alleviated HFD/STZ-induced plasma glucose, triglycerides, cholesterol, % of HbA1c, hepatic steatosis, NLRP3, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain, CARD, caspase-1, IL1β, NFκB, Sod 1,

Cat, IGF 1, heme oxygenase 1, receptor for the advanced glycation end product (RAGE), and collagen-1 in diabetic rats [432].

Kim et al. (2019) have used a sham surgery approach in NRF2-KO male or female rats to examine the effects of hepatic ischemia-reperfusion injury. By correlation of the level of bile transporters expression with the Suzuki score, the serum levels of aminotransferase, bilirubin, and bile acids, and tissue ATP level, they indicated that short warm ischemia time upregulates the transcriptional activities of bile transporter, whereas long warm ischemia time inhibited them, and the effect was blunted by NRF2-KO status [433].

The relationship of NRF2 and vascular endothelial growth factor (VEGF) in angiogenesis was analyzed comparing siRNA-mediated NRF2 knockdown and wild-type using vascular hypertension rat models. SiRNA-targeting NRF2 was injected intraparenchymally in the scull of the rats. Silencing NRF2 prevented the upregulation of the mRNA and protein levels of critical angiogenesis mediators, such as VEGF and HIF-1α induced by hypertension, supporting the role of NRF2 and VEGF interplay in hypertension [434]. A recent study using a KO rat model (SD-Nfe212em1Mcwi) was designed to evaluate the activation of NRF2 in hyperoxia-induced acute lung injury. This study supports the idea that NRF2 provides some protection against lung injury in rats, as reflected by increases in lung expression of glutathione synthetase, 3-nitrotyrosine (index of oxidative stress), and interleukin-1β in wild types compared to NRF2-KO rats. But, on the other hand, there was no significant difference for myeloperoxidase, Bcl-2 or peroxiredoxin-1 expression between wild-type and KO rats [435].

NRF2 activation has been tested in response to dexmedetomidine, highly selective α2 adrenoceptor agonist approved as analgesic by the FDA, on acetic acid-induced acute inflammatory visceral pain in rats. Rats treated with dexmedetomidine exhibited a significant reduction of the protein levels of KEAP1, accordingly NRF2 and HO1 protein levels were increased compared to model group. Interestingly, *KO* of NRF2 did not affect KEAP1 expression, but prevented activation of HO-1 induced by dexmedetomidine in spinal cords [436]. The therapeutic effect of quercetin on liver damage induced by hyperthyroid dysfunction was assessed comparing NRF2-null rats vs. wild type littermates. Administration of T4 hormone increased the levels of serum triiodothyronine and thyroxine, activity of oxidative stress markers with a parallel decrease in antioxidant markers and NRF2. However, the simultaneous administration of quercetin reversed all these effects indicating its potential in the regulation of hyperthyroidism. Furthermore, the loss of NRF2 diminished these effects resulting from the quercetin application, indicating the inhibitory effects caused by the quercetin may be mediated by the NRF2 pathway [437]. A recent paper on NRF2 silencing in rats has confirmed the hepatoprotective effects of oleanolic acid on ANIT-induced cholestatic liver damage in NRF2-KO and additionally Fxr KD rats [438].

In addition, rat models have been used to test the NRF2 role in other NCDs including cardiovascular dysfunction. The compound tanshinol borneol ester (DBZ) inhibited oxidative stress and ER stress, blocked autophagic flow and decreased apoptosis through enhanced NRF2 stability via regulating the mTOR/β-TrCP/NRF2 signaling pathway in a hypertrophic model in rats using TAC surgery and in neonatal rat cardiomyocytes using Ang II [439]. Moreover, a rat model of intense stress (social defeat, first hit) seed light in mechanisms involved in depression vulnerability, since 40 % of the animals developed a depression-like phenotype after a second stressful hit (chronic mild stress). Vulnerable animals had a persistent state of oxidative stress that could be reverted by the NRF2 inducer terbutylhydroquinone [440]. The increased oxidative stress status of vulnerable animals was related to reduced levels of brain derived neurotrophic factor (BDNF). Interestingly, BDNF constitutively controlled the nuclear translocation of NRF2. In contrast, in the report by Yao et al., 2021 [441] they conclude that NRF2 activation increases BDNF. These findings provide a link between reduced BDNF levels, oxidative stress and NRF2 in depression and further support that NRF2 induction as a possible strategy for the treatment of depression. The role of NRF2 in retinal pathologies has also been analyzed by Mandal et al. [442] using a rat model of light-induced retinal degeneration, curcumin reduces retinal oxidative and inflammatory stress and up-regulates the cytoprotective machinery, protecting the retina from intense apoptotic insult. Thus, curcumin could be an effective nutraceutical compound for preventive and augmentative therapy of AMD. There is an ongoing clinical trial studying the effect of oral curcumin supplementation on choriocapillaris and drusen characteristics measured by multimodal retinal imaging in dry AMD patients (NCT04590196). Using the kainic acid-induced status epilepticus model in rats, it has been shown that pharmacological activation of NRF2 by omaveloxolone, administered only during the first week after disease onset, increases the levels of ATP, protects against neuronal death, and reduces the frequency of late spontaneous seizures for at least 4 months following status epilepticus [443].

Especially relevant in chronic diseases in which ageing could be a driving force, is the fact that the naked mole rat (*Heterocephalus glaber*) exhibits high NRF2 binding activity to ARE in liver samples, along with a lifespan four times longer than similarly sized mouse. Surprisingly, it has been described that species longevity was not linked to the protein levels of NRF2 itself, but rather showed a significant negative relationship with the regulators KEAP1 and βTrCP [444,445].

4.6. *Rabbit and pig models (animalia chordata mammalia Lagomorpha and Sus domesticus)*

Even though murine models are frequently used in biomedical research, there are concerns and difficulties because there is less similarity between mice and humans in terms of the pathological mechanisms underlying diseases and medical safety. Thus, there is a growing interest in the possibility of using other animals, which are more closely related to humans.

NRF2 has been a subject of investigation of different diseases in rabbits. Namely, acute respiratory distress syndrome (ARDS) in rabbits was significantly inhibited by SFN-induced upregulation of NRF2 [446], while electroacupuncture treatment effectively attenuates endotoxin-induced ARDS through activation of NRF2/ARE pathway and following up-regulation of HO-1 expression [447]. Mycotoxines also induce oxidative damage and inflammation, increasing hepatocyte apoptosis and liver injury in rabbits. In this context, it was found that selenomethionine pretreatment alleviated intestinal aflatoxin B1-induced oxidative damage and the inflammatory response by activating NRF2/HO-1 in rabbits [448]. Moreover, quercetagetin (natural flavonol compound) alleviated the mycotoxin-induced oxidative damage and liver injury via NRF2 [449]. The levels of NRF2 are increasingly expressed in a parallel time course to the development of cerebral vasospasm in a rabbit experimental model of subarachnoid hemorrhage [450].

Pigs are thought to be a better model than mice because of their greater similarity to human body size, anatomical features, organ size and structure, physiology, and pathophysiology [451]. For instance, the size, shape, and blood supply of their pancreas are comparable to those of the human pancreas [452], and the islet size and ratio among the endocrine cell types in pigs are also comparable to those of humans [453]. Many plant-derived polyphenols act as NRF2 activators, protect against mycotoxin-induced oxidative stress, and promote the intestinal antioxidant capacity of pigs *in vivo*. Among them are resveratrol, ferulic acid, chlorogenic acid, and baicalin, which are known to activate NRF2 and protect against oxidative stress injury in pigs [454]. Ellagic acid (EA) is also a bioactive polyphenolic compound naturally occurring as secondary metabolite in many fruits which displays strong antioxidant properties. EA supplementation promoted NRF2 nuclear translocation and enhanced HO-1 and NQO1 protein abundances of small intestinal mucosa in mycotoxin-induced intestinal injury in piglets [455]. Also, it was found that dietary apple polyphenols increase the intestinal antioxidant capacity and activate the KEAP1-NRF2 pathway in pig and

IPEC-J2 cell models [456].

Flavonoids exhibit direct antioxidant activities by scavenging of free radicals, inhibiting ROS and proinflammatory cytokine productions, and several studies have highlighted the NRF2 activation by these natural products. In this sense, it was found that the supplementation of *Eucommia ulmoides* flavones decreased the oxidized glutathione (GSSG) levels and the ratio of GSSG to reduced glutathione (GSH), but increased the protein levels of nuclear NRF2 and KEAP1, as well as the mRNA expression of HO-1, NQO1, and GCLC in the small intestinal mucosa of herbicide-challenged piglets [457].

Activation of NRF2 has been observed following administration of oxidized oils, which increase gene expression and activities of various enzymes involved in xenobiotic metabolism and stress response. In this sense, Varady et al. (2012) showed time that administration of an oxidized fat activates NRF2 in the liver of pigs [458]. In addition, the transcript levels of antioxidant and phase II genes, such as SOD, HO-1, Trx 1, microsomal glutathione-S-transferase 1 and NQO1 in the liver were higher in the oxidized fat-treated group than in the fresh fat-treated group. In a condition like this, NRF2 activation generally reflects an adaptive mechanism to prevent cellular oxidative damage since it induces the up regulation of numerous antioxidants, cytoprotective, and detoxifying genes and protects the cell from ROS and harmful substances [459].

5. Conclusions and future perspectives

The use of animal models across various evolutionary stages is crucial for understanding the complexity of the molecular mechanisms involving NRF2 and its role in health and disease. These models, ranging from simpler organisms like fruit flies and nematodes to more complex mammals, allow researchers to investigate the conserved pathways regulated by NRF2 and how these pathways contribute to cellular stress responses and overall homeostasis. This comparative approach is essential for identifying the specific roles of NRF2 in non-communicable diseases (NCDs) and determining how its modulation can be effectively harnessed for therapeutic purposes. In fact, research on model organisms has provided an impressive level of understanding of the molecular mechanisms of the role of NRF2 in the pathogenesis of NCDs, leading to more than 100 clinical trials and culminating in the entry of two NRF2 activators, dimethyl fumarate and omaveloxolone, in clinical practice. Further clinical trials with larger sample sizes and longer follow-up periods using NRF2 inducers alone or in combination with classical drugs to palliate symptoms are necessary to test the effectiveness of NRF2 induction in NCDs, especially in those patients that are refractory to treatment. Although the use of model organisms has generated a wealth of essential knowledge, there are still many challenges that need to be overcome for the future development of NRF2 modulators as therapies for NCDs.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Corrigendum to "Model organisms for investigating the functional involvement of NRF2 in non-communicable diseases" [Redox Biol. 79 (2025) 103464]

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The authors regret to inform that a duplicate figure was included in the original publication. The correct version of Figure 9 is available below.

The authors would like to apologise for any inconvenience caused.