Impact of NQO1 dysregulation in CNS disorders

Li Yuhan[1,2], Maryam Khaleghi Ghadiri[3] and Ali Gorji[1,3,4,5]*

Abstract

NAD(P)H Quinone Dehydrogenase 1 (NQO1) plays a pivotal role in the regulation of neuronal function and synaptic plasticity, cellular adaptation to oxidative stress, neuroinflammatory and degenerative processes, and tumorigenesis in the central nervous system (CNS). Impairment of the NQO1 activity in the CNS can result in abnormal neurotransmitter release and clearance, increased oxidative stress, and aggravated cellular injury/death. Furthermore, it can cause disturbances in neural circuit function and synaptic neurotransmission. The abnormalities of NQO1 enzyme activity have been linked to the pathophysiological mechanisms of multiple neurological disorders, including Parkinson's disease, Alzheimer's disease, epilepsy, multiple sclerosis, cerebrovascular disease, traumatic brain injury, and brain malignancy. NQO1 contributes to various dimensions of tumorigenesis and treatment response in various brain tumors. The precise mechanisms through which abnormalities in NQO1 function contribute to these neurological disorders continue to be a subject of ongoing research. Building upon the existing knowledge, the present study reviews current investigations describing the role of NQO1 dysregulations in various neurological disorders. This study emphasizes the potential of NQO1 as a biomarker in diagnostic and prognostic approaches, as well as its suitability as a target for drug development strategies in neurological disorders.

Keywords Brain, CNS disorders, Inflammation, Neurodegeneration, Neuronal excitability, NQO1, Biomarker

Introduction

NAD(P)H quinone dehydrogenase 1 (NQO1) is an enzyme involved in cellular detoxification and protection against oxidative stress. NQO1belongs to the quinone oxidoreductase family and is found in different tissues, including the lung, thyroid, colon, heart, kidney, liver, cornea, and lens, as well as the peripheral and central nervous systems (CNS) [1]. Disruption of the oxidant/antioxidant balance in the CNS exerts significant effects on various physiological processes and contributes to the evolution of several acute and chronic neurological disorders. NQO1 participates in the cell protection process against unwanted oxidative damage and maintains cellular homeostasis through the reduction of free radicals and detoxifying deleterious quinones as well as the modulation of antioxidant genes [2, 3].

Furthermore, NQO1 exerts both direct and indirect modulation over the activity of various signaling pathways, including those that influence cell proliferation [4, 5], apoptosis [6], and neuroinflammation [7]. In the CNS, NQO1 governs the inflammatory responses via different mechanisms, including the modulation of particular pro-inflammatory cytokines and activation of CNS immune cells, like astrocytes and microglial cells [8]. NQO1 contributes to mitigating inflammation in various physiological and pathological conditions through the reduction of oxidative damage and quenching of oxidative stresses

*Correspondence:
Ali Gorji
gorjial@uni-muenster.de
[1] Epilepsy Research Center, Münster University, Münster, Germany
[2] Department of Breast Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[3] Department of Neurosurgery, Münster University, Münster, Germany
[4] Shefa Neuroscience Research Center, Khatam Alanbia Hospital, Tehran, Iran
[5] Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 2 of 28

(ROS). On the other hand, several factors impact the expression and function of NQO1 in the CNS, including oxidative stress, hormonal signaling pathways, and epigenetic modifications.

Different polymorphisms in the NQO1 gene influence the translation of the NQO1 protein and contribute to the occurrence of various neurological diseases. For instance, the C609T polymorphism exerts an influence on various neurodegenerative and neuroinflammatory disorders, such as Alzheimer´s disease (AD) and multiple sclerosis (MS) [9, 10]. Here, we first summarized the current knowledge of the modulatory mechanisms of NQO1 in the CNS. Moreover, we provided a comprehensive review of the current experimental and clinical knowledge of the potential prognostic, diagnostic, and therapeutic roles of the NQO1 in different neurological disorders. Finally, we discussed the challenges in designing and developing novel substances that target NQO1 for diagnostic, preventive, and therapeutic purposes in neurological disorders. Understanding the function of NQO1 and its modulatory role in the CNS is crucial for revealing its potential therapeutic implications in various neurological disorders [11].

General aspects of NQO1
Structure
NQO1 is a flavoprotein that acts as an enzyme and is composed of a NAD(P)H-binding domain and a quinone-binding domain that is encoded by the NQO1 gene [12, 13]. This ubiquitous cytosolic enzyme consists of two uniform subunits, with a dimer molecular weight of about 52 kDa. The exact molecular weight of NQO1 may vary slightly depending on different factors, like tissue source, post-translational modifications, and measurement techniques. The NQO1 gene is located on chromosome 16q22.1 and the sequence of the NQO1 gene can vary between individuals due to genetic polymorphisms. The human NQO1 gene is approximately 20 kb and composed of 6 exons that are interspersed by 5 introns [14]. NQO1 tightly binds flavin adenine dinucleotide (FAD; as a cofactor), which plays an important role in the stability and activity of the enzyme [15]. FAD is involved in the formation of homodimers and plays a role in the reduction of two-electron of quinones to form hydroquinones [16]. NQO1 inhibitors exert their effects through the binding to the NAD(P)H and consequently obstructing the transfer of electrons to FAD [17], particularly through modulation of Tyr 128 and Phe 232 on the surface of the active site of the enzyme [18]. Under physiological conditions, NQO1 is primarily a cytosolic enzyme, but a smaller quantity of NQO1 has been also detected in the nucleus [19].

Functions
Various forms of stress, including oxidative stress, stimulate the bioactivity of NQO1, which plays a crucial role in safeguarding cells against damage and preventing cell death. NQO1 is a cytoprotective antioxidant substance that regulates ROS. NQO1 reduces toxic quinines to form hydroquinone via a single-step two-electron reduction reaction by bypassing the formation of the highly reactive semiquinone [20]. Semiquinone produces ROS through the redox cycle, and the accumulation of ROS results in cell damage and cell death [21]. Therefore, NOQ1 degrades quinones and prevents massive accumulation of ROS and DNA damage from oxidative insults. Experiments using purified recombinant human NQO1 and cancerous as well as non-cancerous cells have shown that NQO1 can also use the electrons provided by NADH or NAD(P)H to react with quinone compounds, directly donating quinone double electrons for the reduction reaction, avoiding the formation of semiquinone, a metabolic intermediate that is carcinogenic and teratogenic, and replacing it with a direct reduction to hydroquinones, and then convert hydroquinones into water-soluble compounds to be excreted from the body, reducing the cytotoxic reaction [22–25]. NQO1 plays a role in the maintenance of several endogenous antioxidants. Studies on the cellular distribution pattern of NQO1 in MS brains have revealed that alterations in NQO1 levels can play a role as a marker for cellular oxidative stress [26]. In experiments with purified human NQO1, it has been shown that NQO1 interacts with α-tocopherol quinone, generated at slow levels during the antioxidant action of vitamin E, to form α-tocopherol hydroquinone and protects against lipid peroxidation of the cell membrane [22, 23]. At relatively high levels of NQO1 in the cells and low values of superoxide dismutase, NQO1 restores α-tocopherol to its hydroquinone form to protect the membranes against lipid peroxidation [27, 28]. Furthermore, studies on fractionated mouse liver extracts and human colon carcinoma cells have revealed that NQO1 can directly reduce superoxide and modulate the degradation of some proteins, such as P53 [19, 29]. NQO1 also indirectly reduces ROS via the changes of different antioxidants, such as vitamin E and coenzyme Q10 [3]. Besides, NQO1 is inhibited by several anticoagulants, such as dicumarol, and strongly decreases vitamin K metabolism [30]. Moreover, investigations on various human cancer cells revealed that NQO1 directly interferes with the unstructured DNA-binding domain of c-Fos and leads to the upregulation of cyclin-dependent kinase subunit-1 and the modulation of the cell-cycle progression at the G2/M phase [5]. NQO1 catalyzes the reduction of membrane-bound coenzyme Q (a ubiquinone analog) to their ubiquinol forms in liposomes and

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 3 of 28

protects membrane phospholipids from oxidative damage [31]. Unrelated to its enzymatic activity, there are cytoprotective roles for NQO1 through several mechanisms, such as the activation of apoptosis. NQO1 binds the tumor suppressor protein p53 and enhances its stability through the inhibition of proteasomal degradation [32]. In addition, the evaluation of vascular endothelial and smooth muscle cell redox signaling revealed that the nuclear factor erythroid 2-related factor 2 (Nrf2)-NQO1 pathway controls cell injury/death through the maintaining of lipid peroxidation and the synthesis of 4-hydroxynonenal, a lipid hydroperoxide end product [33]. It has been demonstrated that the suppression of receptor-interacting serine/threonine-protein kinase 1 inhibits cell death pathways (such as necroptosis and apoptosis) and leads to the suppression of NQO1 activity and NQO1-dependent cell death in various cell lines [34].

Moreover, NQO1 has been shown to modulate the redox state of various transcription factors, such as p53, AP-1, and Nrf2, thereby influencing the expression of diverse genes implicated in cell cycle control, apoptosis, and antioxidant defense. NQO1 has been shown to stabilize p53, a tumor suppressor protein, through a redox mechanism involving the oxidation of NAD(P)H [32, 35]. The interaction between NQO1 and p53 can vary among different cell types, potentially due to the influence of other p53-interacting proteins that dominate in particular cellular contexts [36]. Furthermore, the effect of NQO1 on P53 has been evaluated in in both endothelial cells and various cancer cell types, such as human colon carcinoma cell. NQO1 protects p53 and some other proteins from protease degradation through protein–protein bidirectional interactions [35, 37, 38]. Furthermore, NQO1 modulates the stability of other proteins involved in regulating cell growth and cell death, such as p73 and p33ING1b [3, 5, 28, 39–43]. C/EBPβ (CCAAT/enhancer binding protein β) is a group of transcription factors that play a multifaceted role in diverse cellular processes, such as proliferation, differentiation, and immune response regulation. Studies on different in vitro experimental models suggest that NQO1 and C/EBP may exert bidirectional regulatory interactions on each other and modulate cell proliferation and differentiation in some neurological disorders [44]. NQO1 also interferes with Nrf2 and the enhancer sequence of the antioxidant response element (ARE) [45–50].

NQO1 interaction with mRNA can play a role in cell defense against ROS, malignancy, and metabolic stress by alterations in gene expression, protein synthesis, and cellular function [51]. Using cellular and animal models of cardiac hypertrophy, it has been shown that NQO1 can modulate the stability of mRNA by binding to specific sequences within the mRNA molecule [52]. Furthermore,

several cell culture investigations have shown that NQO1 can directly influence the translation of mRNA into protein by interfering with the ribosome or other components of the translation machinery [53, 54]. A meta-analysis of NQO1 and ribosome subunit gene expression in brain specimens from patients with schizophrenia and controls (overall 511 samples) revealed a positive correlation between NQO1 and upregulated ribosome subunit genes, suggesting that the upregulation of ribosome subunits may lead to altered mRNA translation and disruptions in neuronal network functions [55].

Polymorphism

The locus of the human NQO1 gene exhibits high polymorphism, and different variations have been reported, particularly in the promoter area [56]. NQO1 polymorphism can lead to decreased enzyme activity and stability, leading to increased sensitivity to oxidative stress. Multiple polymorphisms in the NQO1 gene have been associated with altered enzyme activity, which has implications for various health conditions, including cancer, cardiovascular diseases, and neurological disorders. The relationship between NQO1 polymorphisms and neurological disorders is complex and depends on the specific polymorphism and the genetic background of patients [57]. NQO1 polymorphisms are associated with an increased risk of some neurological disorders, such as MS and AD, likely due to structural and functional alterations of NQO1 and disruption in the cellular antioxidant defence [58]. Two main polymorphisms of NQO1 in humans are C609T and C465T. The most influential NQO1 polymorphism is the C609T polymorphism (known also as polymorphism P187S or NQO1*2), which significantly increases the susceptibility for developing various malignancies, such as hepatocellular, bladder, and pulmonary cancers [24, 25, 39, 59–70]. A meta-analysis involving 92 studies, which included 21,178 patients with various types of cancer and 25,157 healthy controls, indicates the implication of C609T polymorphism of the NQO1 gene as a genetic risk factor in different cancers [71]. The C609T polymorphism leads to a proline-to-serine change at codon 187 of the protein, which markedly decreases quinine reductase activity and enzyme stability [70]. NQO1 polymorphism C609T is also associated with enhanced risk of different neurological diseases, such as AD, MS, and Parkinson's disease (PD), presumably via destabilization of FAD affinity and enzyme stability [72]. In a meta-analysis of five case–control investigations involving 735 patients with AD and 828 controls, an association between the NQO1 C609T polymorphism and AD in Chinese populations has been demonstrated [9]. Furthermore, the C609T polymorphism impacts cell redox metabolism as well as cell sensitivity to different

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 4 of 28

therapeutic approaches [73]. This polymorphism influences the response to chemotherapy and radiotherapy in patients with cancer and could be used as a potential prognostic biomarker for cancer therapy resistance [74]. Furthermore, the NQO1 C465T polymorphism (known also as NQO1*3) is a single-nucleotide alteration at nucleotide 465, where a cytosine is replaced by a thymine base, and leads to a marked reduction of enzyme activity [75]. This polymorphism is also associated with an increase in the risk for various cancers [76, 77]. For instance, a meta-analysis that included 28 relevant studies involving 5,953 patients with acute leukemia and 8,667 controls has shown an increased risk of cancer for carriers of the NQO1 C609T polymorphism [77]. The 3423G is another NQO1 polymorphism, which has been accompanied by decreased enzyme activity and enhanced risk of cancer [78]. The frequency and distribution of different NQO1 polymorphisms vary among various ethnic and racial populations [71, 79, 80]. Considering genotype–phenotype correlations in the multifunctionality of the NQO1 protein, genetic diversity, and the influence of post-translational modifications, NQO1 emerges as an exemplary model of a disease-associated antioxidant protein [81]. Furthermore, various therapies designed to correct the stability and dynamics of NQO1 polymorphic proteins and to regulate intracellular factors leading to loss-of-function are proposed as promising approaches for achieving better outcomes [17]. Examining epigenetic modifications on the NQO1 gene and their potential interactions with these polymorphisms could represent an innovative research approach [82].

NQO1: the regulatory pathways
### Nrf2-KEAP1-ARE-NQO1 signaling pathway
The NQO1-Kelch-like ECH-associated protein1 (KEAP1)-Nrf2-ARE signaling pathway involves a series of molecular interactions that contribute to the adaptive cellular stress response, particularly to chemicals that are electrophilic or oxidative in nature [83]. Increasing evidence suggests the protective role of the Nrf2-ARE signal pathway in central nervous diseases mediated by heme oxygenase-1 (HO-1) and NQO1 [84, 85]. The activation of the NQO1 gene occurs in conjunction with other Nrf2-induced detoxifying enzyme genes, including glutathione S-transferase (GST) and HO-1. This activation is triggered by various stimuli, such as free radical scavengers, hypoxic insults, ionizing electromagnetic radiation, chemical substances, heat shock, and heavy metals [86, 87]. Activation of the transcription factor Nrf2 has been shown to provide protection against the harmful effect of excessive oxidative stress in various pathological conditions [1]. Moreover, Nrf2 activation enhances the availability of substrates for mitochondrial function

and promotes adenosine triphosphate (ATP) production, which can have a cell-protective effect [88, 89]. Overexpression of Nrf2 significantly enhances the expression of NQO1 and exerts a strong neuroprotective effect on neurons and neuroglial cells [90].

The KEAP1-Nrf2 interaction plays a key role in preserving cellular balance by detecting oxidative stress and activating the necessary defense mechanisms. Under the physiological state, Nrf2 is subject to proteasome-dependent degradation by KEAP1. Nrf2 undergoes constant ubiquitination by KEAP1, leading to its degradation in the proteasome. Under non-stress conditions, this process promotes the ongoing degradation of Nrf2 protein within the cell (Fig. 1). The interaction between KEAP1 and Nrf2 plays a role as a sensor for oxidative stress. After exposure to oxidative stress (i.e., following hypoxia or inflammation), KEAP1 undergoes a conformational alteration, which prevents it from binding to Nrf2 and leads to the release, accumulation, and translocation of Nrf2 into the nucleus [1, 91] (Fig. 2). Nrf2 interacts with ARE in the promoter area of target genes in the nucleus [83]. Nrf2-ARE binding upregulates the expression of various genes by the enhancement of both transcription and translation. In the case of NQO1, Nrf2 binding to the ARE sequence located in the promoter region of the NQO1 gene leads to its increased expression [90, 92]. After NQO1 transcription, it is translated into an enzyme that helps to prevent the generation of ROS. Through its regulatory function, Nrf2 plays an essential role in enhancing the expression of NQO1 and other genes involved in the cellular defense pathway against the detrimental effects of oxidative stress (Fig. 2) [1, 92].

Furthermore, AMP-activated protein kinase (AMPK) has been revealed to activate the Nrf2-ARE-NQO1 signaling axis. Activation of AMPK leads to the phosphorylation of Nrf2 and promotes its nuclear translocation and subsequently leads to the activation of ARE-driven genes, including NQO1 [93]. This procedure enhances antioxidant enzyme levels and protects cells from oxidative stress [94]. Besides, the activation of AMPK-NQO1 signaling has been reported to modulate cellular metabolism, boost mitochondrial function, and augment ATP production, which may also contribute to neuroprotection [95]. NQO1 also plays a crucial role in regulating inflammatory responses via its modulation of AMPK and downstream signaling pathways [7]. Using in vitro cell line experiments, it has been proposed that the activation of Nrf2-KEAP1-antioxidant response element-NQO1 signaling pathway could inhibit apoptosis and protect tissues from hypoxic insults [96]. The therapeutic potential of modulating this pathway might be a promising option for future studies.

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 5 of 28
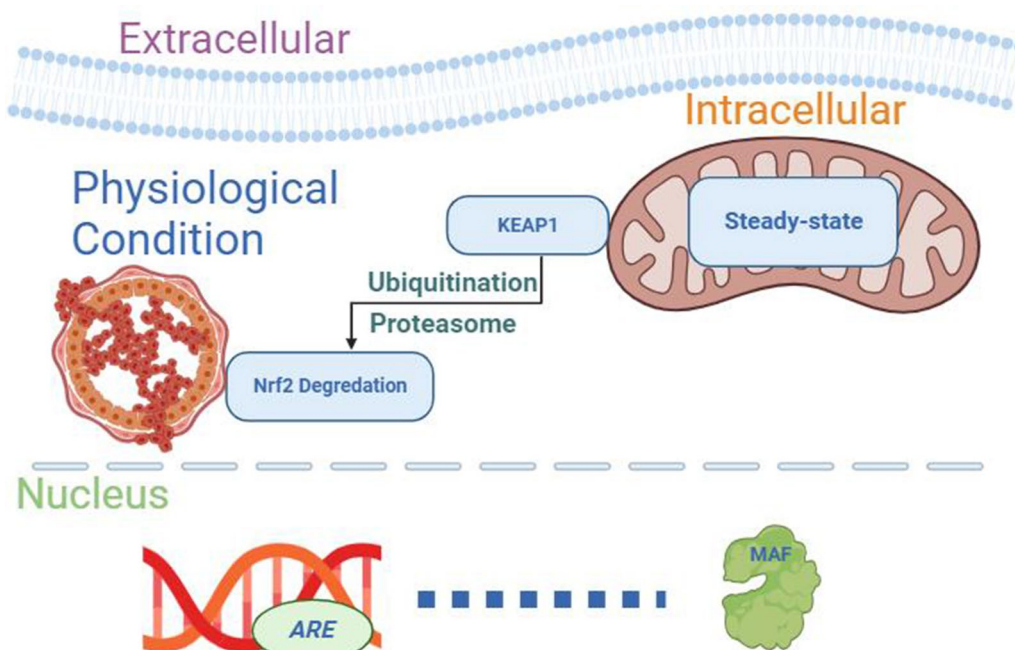
Fig. 1 Schematic depiction of the induction and functions of the Nrf2-NQO1 pathway in physiological conditions. In a steady-state condition, erythroid 2-related factor 2 (Nrf2) is sequestered within the cytosol by the repressor protein Kelch-like ECH-associated protein1 (KEAP1). This is crucial for KEAP1 to remain unaffected by external factors, preserving its original conformation. This stability ensures the natural degradation of Nrf2 and prevents Nrf2 from entering the nucleus and binding to the antioxidant response element (ARE). This process avoids the forming of heterodimers with small MAF proteins and activating the antioxidant genes. Maintaining the stability of this pathway relies on preventing Nrf2 from entering the nucleus and enabling its natural degradation within the cell [1–3, 91]

CREB-Nrf2/HO-1/NQO1 signaling pathway

The activation of the cAMP response element-binding protein (CREB) increases the expression of Nrf2 [97], which then enhances the expression of NQO1 via the upregulation of ARE. Moreover, the activation of the CREB signaling increases the expression of hypoxia-inducible factor 1-alpha (HIF-1α), an oxygen-dependent transcriptional activator. Consequently, HIF-1α has been shown to upregulate NQO1 expression in response to stressful events, such as hypoxia. Activation of CREB enhances the transcriptional activation of HO-1 in neurons in response to tissue injuries-induced oxidative stress, such as malignancy or hypoxia [98]. This pathway may represent a novel target through which the inflammatory disorders can be therapeutically conditioned [98]. There are bidirectional interactions between NQO1 and HO-1, where the activation of one protein can modulate the expression of the other. NQO1 promotes HO-1 expression through the activation of Nrf2 and upregulation of HO-1, in turn, can further enhance NQO1 expression [99].

NQO1-Nrf2-PI3K/Akt pathway

The activation of the phosphoinositide 3-kinase (PI3K)/Akt signaling has been shown to increase the expression of NQO1 in several cell types, including pheochromocytoma cells and cancer cells [100, 101]. The PI3K/Akt pathway activation plays an important role in enhancing cell survival and growth through the phosphorylation of a variety of downstream targets involved in cell proliferation, metabolism, and apoptosis [102–105]. The activation of the PI3K/Akt signaling also promotes the detachment of Nrf2 from KEAP1, facilitates its translocation to the nucleus, and regulates Nrf2 activation in a ROS-dependent manner [106, 107]. The induction of the PI3K/Akt/Nrf2 signaling axis ameliorates oxidative stress and apoptosis via the regulation of Nrf2, NQO1, and HO-1 [108].

The interaction between the CREB-Nrf2 /HO-1/NQO1, Nrf2-KEAP1-ARE-NQO1, and NQO1-Nrf2-PI3K/Akt pathways implicates different and complex cellular processes linked directly and indirectly to the oxidative stress response. These pathways are interconnected, but their specific interactions in both physiological and pathological conditions depend on the specific cellular microenvironmental conditions [109, 110]. The regulatory effects of the interaction between these pathways on cellular metabolism and their role in addressing oxidative stress could be a potential shared therapeutic target for the treatment of various disorders, such as
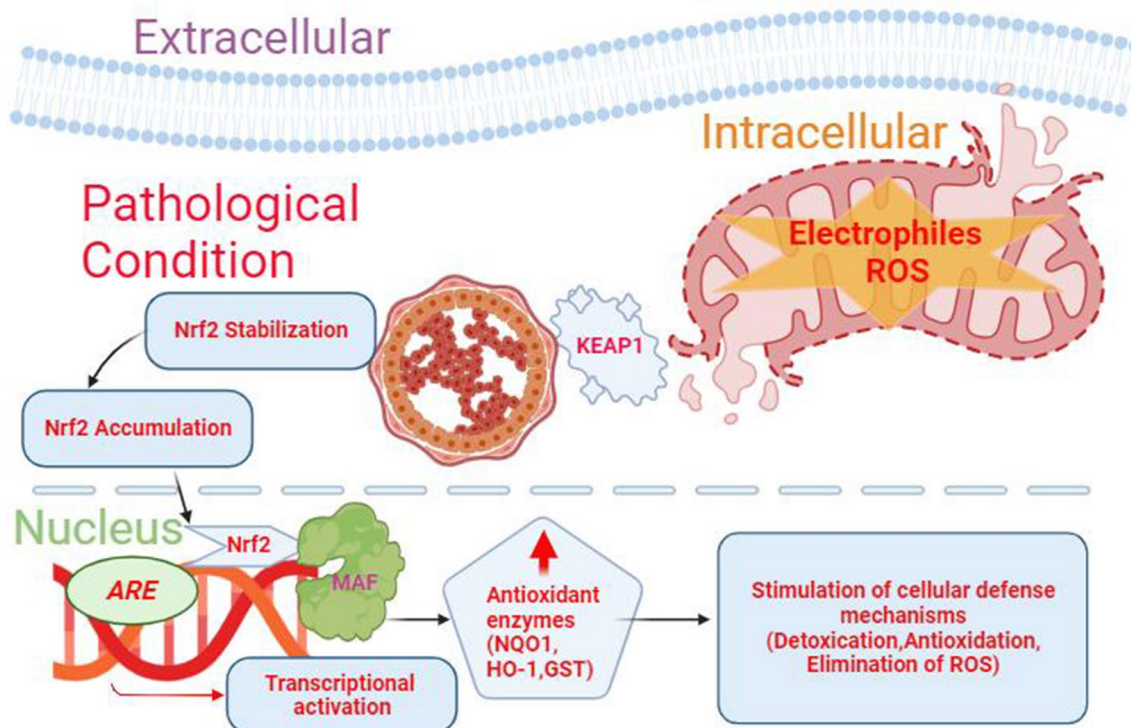
Fig. 2 Schematic illustration of the initiation and roles of the Nrf2-NQO1 pathway in pathological conditions. During conditions of oxidative stress, reactive oxygen/nitrogen species (ROS) react with Kelch-like ECH-associated protein1 (KEAP1), leading to a conformational change that accumulates and releases nuclear factor erythroid 2-related factor 2 (Nrf2), preventing its degradation. Furthermore, small molecules can directly inhibit the Nrf2-KEAP1 interaction, resulting in the liberation of Nrf2. Consequently, Nrf2 translocates into the nucleus and forms heterodimers with small Maf proteins, binding to antioxidant response element enhancer (ARE) sequence and initiating the transcription of antioxidant enzymes, such as NAD(P)H Quinone Dehydrogenase 1 (NQO1), heme oxygenase-1 (HO-1), and glutathione S-transferase (GST). This triggers the activation of diverse cellular defense mechanisms and initiates the activation of numerous anti-oxidative stress genes. This coordinated response serves as a protective mechanism against cell damage and could be used as a therapeutic approach for various pathologies, including those affecting the central nervous system [1–3, 92]

cancer, autoimmune disorders, and neurodegenerative diseases (Fig. 3) [111, 112].

The involvement of NQO1 in neurological disorders

NQO1 is expressed in various cells within the CNS [113]. NQO1 is predominately expressed in astrocytes and a subgroup of oligodendrocytes, but lower levels of NQO1 are also detected in neuronal cell bodies, dendrites, and synapses [113, 114]. Numerous investigations have evaluated the potential role of NQO1 in the development and progression of various neurological disorders. These experimental and clinical assessments indicate that NQO1 may be implicated in the pathophysiology of a wide range of both acute and chronic neurological disorders, including epilepsy, AD, PD, amyotrophic lateral sclerosis (ALS), MS, cerebrovascular diseases, and brain malignant tumors (Table 1) (Fig. 4) [115].

NQO1 and AD

Numerous pre-clinical and clinical studies indicate that NQO1 may play an important role in the evolution of AD. These investigations suggest that targeting NQO1 represents a potentially effective method for the treatment of AD. As previously discussed, NQO1 plays a neuroprotective role against oxidative stress and inflammation in the brain, which are well-known processes in the pathophysiology of AD. In an experimental AD mice model, the expression of NQO1 significantly increased in the hippocampus and neocortex in the initial stages of AD, which was followed by a progressive decline during the further development of AD-like pathology in comparison to wild-type mice [116]. Long-term administration of Nrf2 inducer enhances NQO1 expression and prevents the onset of cognitive dysfunction by inhibiting oxidative stress and neuroinflammation in a knockdown mouse model of AD [117]. NQO1 has been found to posttranscriptionally regulate numerous genes involved in apoptosis, and massive neuronal death due to apoptosis is a

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 7 of 28
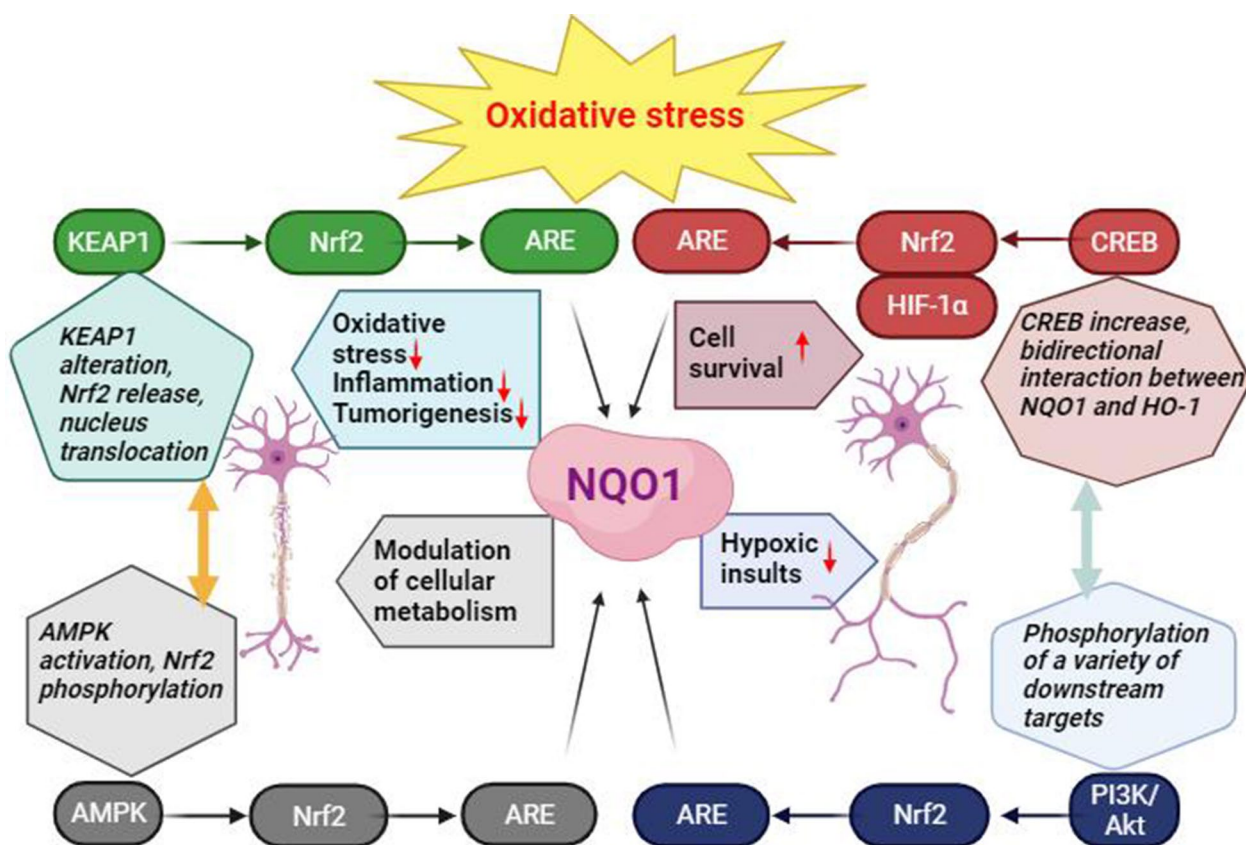
Fig. 3 The interaction between the Nrf2-KEAP1-ARE-NQO1, CREB-Nrf2/HO-1/NQO1, and NQO1-Nrf2-PI3K/Akt signaling pathways plays a crucial role in the regulation of oxidative stress. The interplay between these pathways leads to a coordinated cellular defense against oxidative stress. Following the occurrence of oxidative stress, nuclear factor erythroid 2-related factor 2 (Nrf2) is a key player in the transcriptional regulation of various genes involved in the antioxidant response, including NQO1. The CREB-Nrf2/HO-1/NQO1 pathway can synergistically enhance the expression of antioxidant enzymes when Nrf2 accumulates. Moreover, NQO1 has a bidirectional modulator effect with the PI3K/Akt signaling pathway to further enhancement of the anti-oxidative response. The modulation of the interaction between these pathways on cellular metabolism and their role in addressing oxidative stress could be a potential shared therapeutic target for the treatment of various neurological disorders. NQO1 can well connect various pathways and act as a bridge. It mainly protects nerve cells by resisting oxidative stress, enhancing cell metabolism, promoting cell survival, and preventing hypoxia. Interactions between these pathways may vary in different cell types and under different conditions [84, 97, 109–112]

well-known characteristic of AD pathophysiology [118]. The presence of C609T polymorphism resulted in significantly faster aggregation kinetics of amyloid-β (Aβ)1−42 than with wild-type NQO1. Moreover, the application of dicoumarol, an NQO1 inhibitor that binds in close proximity to the FAD-binding site of the enzyme and stabilizes it, significantly reduced the aggregation kinetics of Aβ1-42 [119]. Using murine melanoma cell line B16, depletion of riboflavin has been shown to induce instability of NQO1 and promote amyloid aggregation in cells [120]. The application of NXPZ-2, a small molecule that disrupts the interaction between KEAP1 and Nrf2 proteins, in an Aβ1-42 oligomer-injected mouse model led to an upregulation of Nrf2 and NQO1 in the whole cell lysate and enhanced nuclear translocation. This

treatment led to decreased Tau values in the hippocampus and neocortex of the AD mice [121]. Treatment with deferiprone (an oral iron chelator) rescued the expression of NQO1, restored memory, and increased hippocampal brain-derived neurotrophic factor (BDNF) levels in an experimental animal model of memory impairment [122]. DHPA, an antioxidant and anti-inflammatory substance, inhibited Aβ1-42/Cu^{2+}/ascorbic acid-induced oxidative damages by regulating mitochondrial apoptosis in human neuroblastoma SH-SY5Y cells, inducing the KEAP1/Nrf2/HO-1 signaling axis and enhancing the expression of NQO1 [123].

Numerous studies indicate that neurons in the substantia nigra (SN) experience persistent oxidative stress. This is supported by observations that both individuals with

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 8 of 28

Table 1 A concise overview of the role of NQO1 in diverse neurological disorders

CNS Disorders	The roles of NQO1 in CNS disorders				Experimental conditions	Methodologies	References
AD	Protective role against oxidative stress and inflammation	Regulation of genes involved in apoptosis	Promotion of amyloid aggregation	Aggregation kinetics of Aβ 1–42	Experimental studies (Human, rats, mouse tissues) Cell culture studies (B16,SH-SY5Y cells, glial cells, pyramidal cells)	Molecular biology, Histopathology, Immunohistochemistry, Behavioral assessments, Biochemical analysis	[72, 116–130]
PD	Protective effect on dopaminergic neurons	Prevent cell death, and modulate neurotrophin receptor affinity in PD	Protect against neurotoxicity induced by MPTP	Impaired function of DJ-1 can lead to impairment of NQO1 function	Experimental studies (Human, mouse tissues) Cell culture studies (Dopamine cells, CATH, SK-N-BE(2)C cells)	Molecular biology, Histopathology, Immunohistochemistry	[41, 131–156]
MS	NQO1 expression is upregulated in active and chronic active MS lesions	NQO1 and Nrf2 levels may be useful as biomarkers	Upregulation of NQO1 effects of dimethyl fumarate in the EAE model of MS	Nrf2 inducers TFM-735, dimethyl fumarate, sulforaphane, and myricetin	Experimental studies (Human, mouse tissues) Cell culture studies (Human primary cells, human brain endothelial cells) Clinical studies (Dimethyl fumarate, Natalizumab)	Molecular biology, Histopathology, Immunohistochemistry, Behavioral assessments	[10, 26, 157–173]
Cerebrovascular disease	Reduces oxidative stress after cerebral ischemia and subarachnoid hemorrhage	Upregulation of NQO1 plays a role in facilitating ischemic preconditioning	Nrf2-ARE-NQO1 pathway contributes to survival of astrocytes and neurons	Nrf2-ARE pathway is activated after subarachnoid hemorrhage (SAH)	Experimental studies (Human, rats, mouse tissues) Cell culture studies (Mouse brain microvascular endothelial cells) Clinical studies (Dimethyl fumarate)	Molecular biology, Histopathology, Immunohistochemistry, Behavioral assessments	[95, 174–201]
Traumatic Brain Injury	Contribution of oxidative stressgene expression process	Decreased neocortex injury, neutrophil infiltration, and microglia activation	As a promising therapeutic strategy	Promoted the nuclear translocation of Nrf2	Experimental studies (Human, rats, mouse tissues) Cell culture studies (Mouse brain microvascular endothelial cells)	Molecular biology, Histopathology, Immunohistochemistry, Enzyme assay, Neurobehavioral evaluation	[202–218]

Table 1 (continued)

CNS Disorders	The roles of NQO1 in CNS disorders		Experimental conditions	Methodologies	References
Epilepsy	Upregulated in hippocampal tissue from individuals with epilepsy	Protecting against seizures and epilepsy-induced brain damage	Experimental studies (Human, rats, mouse tissues) Cell culture studies (Sprague–Dawley rat mixed cortical neurons and glial cells)	Electrophysiological recordings, Molecular biology, Electron microscopy, Histopathology, Immunohistochemistry, Behavioral evaluation	[89, 113, 219–231]
	Mitigating epilepsy and associated comorbidities	Reduces apoptosis and pro-inflammatory cytokines in epilepsy model			
ALS	Protect cells from oxidative stress by detoxifying ROS	Activation of the Nrf2/ARE pathway	Experimental studies (Human, mouse tissues) Cell culture studies (NSC-34,SOD1-G93A cells) Clinical studies (Fasudil, ferulic acid, caffeic acid,)	Molecular biology, Histopathology, Immunohistochemistry, Behavioral evaluation, Biochemical analysis	[232–247]
	Provides neuroprotective effects against neuronal degeneration	Reduce neuronal toxicity			
Brain tumors	Anti-oxidative stress response	Impact on glioma therapy	Experimental studies (Human, mouse tissues) Cell culture studies (SH-SY5Y cells, U87MG, LN229 GBM cells,T98G, LN-229,A172,U251 glioma cells) Clinical studies (Prognostic biomarker)	Molecular biology, Immunohistochemistry, Biochemical analysis, Bioinformatic analysis, Enzyme assay	[3, 44, 50, 59, 72, 248–268]
	Regulating the levels of ROS in glioblastoma	Dual role in glioblastoma development			

NQO1 plays an important role in diverse neurological disorders by the modulation of oxidative stress and cellular redox balance. Its upregulation or downregulation affects the pathophysiology of these neurological disorders. The table describes the complex roles of NQO1 in central nervous system disorders and highlights various experimental conditions and methodologies used by different preclinical and clinical studies. It provides an overview of NQO1's involvement in different neurological conditions, elucidating its impact within experimental settings and clinical investigations. ALS; amyotrophic lateral sclerosis, AD; alzheimer's disease, Aβ; amyloid-β, CNS; central nervous system, MS; multiple sclerosis, NQO1; NAD(P)H Quinone Dehydrogenase 1, PD; Parkinson's disease, ROS; oxidative stresses

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 10 of 28
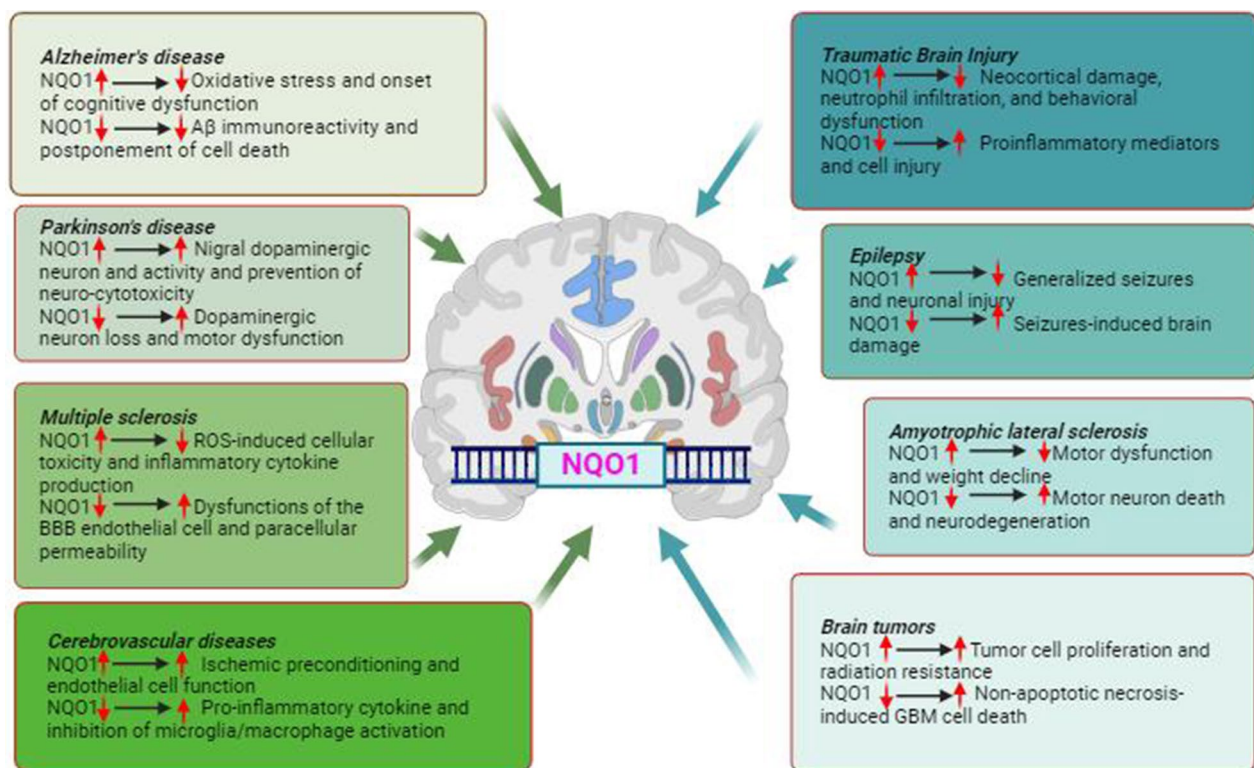
Fig. 4 An overview of the potential effects of NQO1 alterations on the pathophysiology of various neurological disorders. Specific impacts of increased and decreased NQO1 values in each disease are outlined. The changes of NQO1 expression and function may contribute to the pathophysiology of various neurological disorders by affecting the neural tissues to counteract oxidative stress and maintain redox homeostasis. This could potentially lead to the alterations in susceptibility to neurodegeneration and neuroinflammation. The functions of NQO1 changes appear diverse and sometimes conflicting [113–116]

AD and controls have exhibited greater neuronal NQO1 activities in this particular area of the brain [124]. Investigations assessed brain tissues from patients with AD have reported varying results regarding NQO1 alterations. Postmortem studies on hippocampal tissues from patients with AD have shown an enhancement of NQO1 expression in their neurons compared to young and age-matched control autopsies. This investigation has identified two different forms of NQO1 expressions; *i)* NQO1 was detected within intracellular neurofibrillary tangles, neuropil threads, and pre-neurofibrillary tangles, and *ii)* NQO1 was identified as cytoplasmic staining in hippocampal pyramidal neurons [125]. Some studies found NQO1 upregulation in glial cells and later in hippocampal neurons in AD patients, suggesting that increases in NQO1 enzymatic activity may exert a neuroprotective effect [126]. The upregulation of NQO1 in hippocampal pyramidal cells of subjects with AD suggested that NQO1 may be involved in the brain neuroprotective mechanism that is activated in response to the pathological processes of AD [127]. However, another investigation reported that approximately half of patients with AD may lack NQO1 expression in the hippocampus due to a C609T polymorphism [72, 118]. It has been suggested that the age-dependent accumulation of NQO1 may be impaired in individuals with AD. Evaluation of hippocampi from patients with AD revealed that the NQO1 protein level was undetectable in 50% of the cases due to the presence of the C609T polymorphism [127]. AD is a heterogeneous disease and this discrepancy between these studies could be attributed to the broad spectrum of genotype and phenotype as well as various interactions with environmental factors in AD [128]. Moreover, evidence suggests that the NQO1 gene polymorphism C609T may not be an additional genetic risk factor for late-onset AD [129]. Targeting Nrf2 and its downstream genes, including NQO1, could provide potential benefits for the management of AD through the activation of antioxidant genes, the suppression of microglia-mediated inflammation, and the promotion of mitochondrial function [130].

NQO1 and PD
PD, a progressive neurodegenerative condition, is identified by the gradual degeneration of dopaminergic

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 11 of 28

neurons and loss of dopamine (DA) production in the SN [131]. A growing body of evidence suggests that oxidative stress and redox dysfunction play a crucial role in the vulnerability of DA cells to harmful events within the brain and may be involved in the pathophysiology of PD [132]. Several studies have suggested that NQO1 exerts a protective effect on DA cells, presumably by alleviating oxidative stress-induced damages [41, 133, 134]. BDNF may contribute to the pathogenesis of PD by the activation of the Nrf2-ARE-NQO1 signaling pathway, which promotes interactions between astrocytes and neurons, protects dopaminergic neurons from oxidative damage, prevents ferroptosis-induced cell death, and modulates the affinity of neurotrophin receptor [135, 136]. Appropriate activation of NQO1 is dependent on the proper function of DJ-1, a multifunctional protein that regulates cellular metabolism [137]. Loss of DJ-1 was accompanied by a decrease in the stability of Nrf2 pathway activity and deficits in NQO1 expression [138]. NQO1 is expressed in the SN and has been found to be markedly increased in PD patients [139]. The expression values of NQO1 are significantly enhanced in the early and intermediate stages of PD, but decrease in the end-stage of the disease [139]. It has been shown that Akt phosphorylates NQO1, which promotes its ubiquitination and subsequent degradation. Parkin, an E3 ubiquitin ligase, is involved in a procedure that ultimately impacts the antioxidant efficacy of NQO1 in an animal model of PD [140]. Analysis of postmortem specimens from subjects with PD revealed that cytoprotective proteins associated with Nrf2 expressions, such as NQO1 and p62, were partially sequestered within Lewy bodies. This observation suggests a diminished neuroprotective capacity of the Nrf2 signaling pathway in PD [141]. Besides, chlorhexidine, an antimicrobial substance, has been shown to provide cytoprotection in a neurotoxic cell model of PD by disrupting KEAP1-Nrf2, which leads to the nuclear translocation of Nrf2 and subsequent upregulation of downstream genes, including NQO1 [142]. A meta-analysis evaluated the association of NQO1 and tumor necrosis factor (TNF) polymorphisms with PD in data obtained from 15 studies comprising 2,858 patients with PD and 2,907 healthy controls. The findings indicated that TNF-1031 polymorphism may pose a risk for PD under either recessive or additive models. However, the meta-analyses did not support the involvement of NQO1 C609T and TNF-308 in PD risk [143].

MPTP, a neurotoxin, is commonly used to induce PD-like features in rodents [144–146]. The application of MPTP augmented hydroxyl radicals and oxidative stress and enhanced intracellular ROS in the rat SN, which ultimately led to cell death [147, 148]. The activation of NQO1 through the use of sulforaphane, a natural compound with antioxidant and anti-inflammatory properties, can provide protection against MPTP-induced neurocytotoxicity in vivo [149] as well as against 6-hydroxydopamine-induced cell injury in vitro [150]. Several other compounds, such as vinyl sulfoxide and isothiocyanate compound 3, activate the Nrf2-KEAP1 signaling pathway and increase mRNA and protein levels of the Nrf2-dependent antioxidant enzymes, including NQO1, in lipopolysaccharide-activated BV-2 microglial cells [151, 152]. Treatment with the indole derivative NC009-1 improved both motor impairment and depressive behaviors and also increased DA and DA transporter values in the SN. Furthermore, administration of NC009-1 reduced oxidative stress and decreased microglia and astrocyte activity in the ventral midbrain of mice after exposure to MPTP. These beneficial effects were accompanied by an upregulation of Nrf2 and NQO1 as well as a downregulation of iNOS, TNF-α, IL-6, and IL-1β [153]. Moreover, isoliquiritigenin, a flavonoid compound, has been shown to improve motor deficits in a PD mice model through the upregulation of Nrf2 and NQO1, while concurrently downregulating proinflammatory mediators [154]. The application of dimethyl fumarate increased Nrf2 and NQO1 values in the basal ganglia and simultaneously protected dopaminergic neurons from α-SYN-induced toxicity and alleviated microgliosis and astrocytosis in the mouse SN [141]. Although studies have shown that susceptible variants of NQO1 genes could interact with some pesticides and increase PD risk [155], a meta-analysis evaluating sixty-four studies evaluating the relationship between pesticide exposure and PD revealed no significant association between exposure to pesticides and alteration of NQO1 [156]. Further investigation into the modulation of NQO1 function without causing adverse effects and evaluation of additional molecules that interact with NQO1 in the context of PD, particularly on post-mortem specimens or induced pluripotent stem cell-derived neuronal cultures from PD patients, could potentially reveal new therapeutic opportunities.

NQO1 and MS

Oxidative stress is implicated in the onset and progression of MS [157]. NQO1 expression in the CNS of patients with MS may serve as a biomarker of oxidative stress. Both clinical and experimental studies suggest that NQO1 upregulation may indicate an inherent protective response against ROS-induced toxicity in MS [26]. The protein values of NQO1 and Nrf2 were markedly reduced in human brain microvascular endothelial cells following exposure to IgG purified from sera of individuals suffering from myelin oligodendrocyte glycoprotein antibody-associated disorder [158]. The concurrent

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 12 of 28

presence of inflammation and astrocytic NQO1 expression has been observed in patients with MS. Assessment of brain tissues obtained from ten autopsy MS patients as well as seven age and region-matched controls revealed that NQO1 expression was primarily identified in astrocytes located at the center and rim of the chronic active plaque. A direct association was observed between the number of NQO1-expressing astrocytes and the infiltration of T-cells. Moreover, a significant correlation was reported between the mean number of NQO1-expressing astrocytes and astrocytes expressing peroxiredoxins [159]. Evaluation of 64 brain lesions from 14 patients with clinically diagnosed and neuropathologically confirmed MS revealed increased NQO1 expression in macrophages and astrocytes within active and chronically active demyelinated lesions. It has been suggested that NQO1 upregulation in these lesions could be induced by ROS derived from macrophages and serve as a protective mechanism against ROS-induced cellular toxicity [26]. Genetic nuances associated with NQO1could impact could impact the activity or expression of this enzyme and may have implications for an individual's susceptibility to MS. In a case–control investigation involving 231 MS patients and 380 controls, an association between the C609T inborn polymorphism of NQO1 and the risk of developing primary progressive MS has been reported in the Greek population [157]. However, it should be noted that another study conducted on the Spanish Caucasian population did not validate this association [160].

Several studies have demonstrated that the induction of the Nrf2 signaling pathway can suppress the development and progression of disease in various animal MS models [161]. Using human primary cell profiling systems in the experimental autoimmune encephalomyelitis mouse model (EAE) of MS, TFM-735, a potent Nrf2 inducer, has been shown to enhance NQO1 levels in the brain and spleen, inhibit inflammatory cytokine production, and ultimately prevent disease progression [162]. Furthermore, the application of dimethyl fumarate, an antioxidant medicament and Nrf2 inducer approved for the treatment of MS, resulted in a higher expression of the NQO1 in various in vitro and in vivo experimental models [163, 164]. The upregulation of NQO1 is an essential part of the anti-oxidative/inflammation cascade that contributes to the effects of dimethyl fumarate in the EAE model [165]. Furthermore, greater protection of human astrocytes following the application of dimethyl fumarate is associated with the activation of NQO1 [165]. In an in vivo mouse model of MS, sulforaphane, an organosulfur compound, and Nrf2 inducer, has been shown to decrease oxidative stress levels in the brain. This effect is linked to enhanced activation of the Nrf2/ARE pathway, which leads to increased levels of the antioxidant

enzymes HO-1 and NQO1 [166]. Furthermore, melatonin has been shown to suppress lymphocytic infiltration and oxidative stress levels and was associated with a decrease in disease progression in the EAE mouse model. This effect is also thought to be due to the activation of the Nrf2/ARE pathway and the subsequent increase in values of the antioxidant enzymes HO-1 and NQO1 [167]. Myricetin, a flavonoid commonly found in various plants, has been shown to reduce demyelinating lesions by promoting Nrf2 translocation to the nucleus and enhancing the expression of HO-1 and NQO1 in a cuprizone-induced demyelination model of MS [168].

An assessment of the impact of dimethyl fumarate on peripheral immune cells in patients with MS indicated that subjects with higher values of NQO1 after receiving dimethyl fumarate were more likely to have no evidence of disease one year later. Furthermore, the extent of NQO1 induction has shown a correlation with the age of the patient [169]. A statistically significant increase in NQO1 expression was observed in MS patients treated with dimethyl fumarate compared to their baseline levels and placebo-treated patients [170]. A significantly greater frequency of both homozygous and heterozygous NQO1 C609T genotype has been reported among patients suffering from MS compared to controls. MS patients have shown a 1.5-fold enhanced risk of carrying at least one variant of the NQO1 C609T genotype in comparison to healthy individuals [157]. A case–control study involving 254 MS patients and 370 healthy donors has revealed a significantly greater occurrence of NQO1 variant genotypes in patients with relapsing–remitting MS compared with controls, suggesting the role of the NQO1 gene as a risk factor for MS [10]. Evaluation of 130 patients with MS has shown a significantly enhanced frequency of NQO1 mutant polymorphisms documented in nonresponder MS patients following natalizumab administration compared to the responders. Polymorphisms in the NQO1 gene may be a key factor in the treatment of MS patients in the future. Patients who carry the wild-type genotype or only one polymorphism have shown a better clinical outcome following the treatment with natalizumab, a humanized monoclonal antibody used to treat MS [171]. The application of the primary metabolite of dimethyl fumarate, monomethyl fumarate, which is currently used in the clinics for the treatment of relapsing–remitting MS, significantly induced NQO1 in inflamed human brain endothelial cells and decreased monocyte transendothelial migration. These findings suggest beneficial therapeutic effects on the inflamed blood–brain barrier in MS patients [172]. Both dimethyl fumarate and monomethyl fumarate induced NQO1 gene expression in ex vivo-stimulated human peripheral blood mononuclear cells obtained from 200 MS patients

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 13 of 28

treated with dimethyl fumarate [170]. In addition to its role as a therapeutic target, the Nrf2-NQO1 pathway has been proposed as a potential biomarker for predicting and evaluating treatment outcomes in MS [169, 173].

NQO1 and cerebrovascular diseases

Growing evidence suggests a potential association between NQO1 and the pathogenesis of various cerebrovascular diseases, including ischemic stroke and cerebral infarction. The expression of NQO1 is upregulated in response to hypoxia/reoxygenation insults, and this upregulation plays a role in facilitating ischemic preconditioning through the reoxygenation-dependent Nrf2 pathway [174]. The Nrf2-ARE-NQO1 signaling pathway is not only involved in maintaining cerebral blood flow but could contribute to the survival of astrocytes and neurons following cerebral ischemia–reperfusion injury [175]. Several studies have shown that NQO1 expression and activity are altered in animal models of stroke as well as in patients with stroke. A study on 141 patients with ischemic stroke and 139 matched control subjects has shown a significant association between NQO1 gene polymorphisms and the incidence of ischemic stroke. This investigation revealed a correlation between the cc genotype of NQO1 rs1800566 and the occurrence of ischemic stroke [176]. An investigation on the clinical efficacy of butylphthalide in 127 patients with acute ischemic stroke revealed a link between the improvement of the cerebrovascular vascular reserve function and enhancement of the establishment of collateral compensatory vessels with changes in the expression of Nrf2-KEAP1-ARE-NQO1 signaling pathway. Following the administration of butylphthalide in patients with stroke, the serum levels of Keap1 were elevated, while the serum levels of NQO1, Nrf2, and ARE were reduced compared to those in the control group [177]. Simultaneously conducting clinical research (with 100 patients) and an animal study revealed that dimethyl fumarate exerted a neuroprotective effect against post-stroke cognitive impairment through its antioxidative properties. Moreover, dimethyl fumarate improved cognitive impairment induced by ischemic stroke. This study also showed higher expression values of NQO1 in the rat ischemic brain that received dimethyl fumarate in comparison to the Sham group [178]. Furthermore, a case–control study involving postmortem tissues of 26 patients with stroke due to supratentorial intracerebral hemorrhage, it has been shown that NQO1 expression was significantly higher in the peri-hematoma area compared to distant ipsilateral brain tissue obtained 7 days after the onset of hemorrhage [179]. The rats treated with dimethyl fumarate and subjected to a middle cerebral artery occlusion (MCAO) showed enhanced performance in Morris water maze and shuttle box tasks,

as well as a more orderly arrangement of Nissl bodies and neurons, fewer apoptotic cells and autophagosomes, and higher NQO1 expression, compared to the control rats [178]. After MCAO, dimethyl fumarate acts as an effective immunomodulator by decreasing pro-inflammatory cytokines, inhibiting the infiltration of neutrophils and T-cells, and reducing the number of activated microglia/macrophages in the brain infarct zone within 1–2 weeks [180]. In this rat model, the administration of dimethyl fumarate resulted in reduced neurological deficits and infarct size, which was accompanied by increased expression levels of Nrf2, HO-1, and NQO1 [181]. Rev-erbα, a circadian clock protein, plays a complex role in the regulation of inflammation and oxidative stress [182]. Rev-erbα ameliorates acute neurological deficits, reduces infarct volume, and enhances the activation of Nrf2 and its downstream targets HO-1 and NQO1 following an ischemic event in mice [183]. In male rats treated with MCAO and reperfusion, inhibiting Cullin 3, a member of the ubiquitin ligase family, suppressed the ubiquitin-mediated degradation of Nrf2, increased the nuclear translocation of Nrf2, and upregulated the expression of HO-1 and NQO1 [184]. Pharmacological and/or dietary induction of the Nrf2/HO-1/NQO1 pathway may serve as a therapeutic strategy to prevent oxidative stress-induced damage in the peripheral vessels or the blood–brain barrier in stroke [185]. Sulforaphane significantly activated Nrf2 and upregulated mRNA expression of HO-1 and NQO1 in mouse brain microvascular endothelial cells adapted to hyperoxic and normoxic O_2 levels. Inhibition of Nrf2 transcription in these cells led to the loss of HO-1 and NQO1 and a significant increase in free radical generation upon reoxygenation [186]. The induction of the Nrf2-ARE-NQO1 pathway by sulforaphane additionally provides protection to developing neurons and astrocytes against cell death induced by ischemic injuries [187].

In a rat model of cerebral ischemia, the inhibition of mitochondrial dihydrolipoamide dehydrogenase, a potential target for protection against ischemic insults, resulted in the upregulation of NQO1 expression. This intervention led to a reduction in oxidative stress, a decrease in cell injury/death, and an increase in mitochondrial ATP output [188]. The application of an enriched micromilieu, an environment that is designed to provide increased cognitive, sensory, and motor stimulation, has been shown to be an effective approach for improving cognitive dysfunction in cerebrovascular diseases [189]. An enriched environment has been shown to enhance cognitive function in rats with post-stroke cognitive impairment through the suppression of neuroinflammation and oxidative stress. This effect was accompanied by the upregulation of mRNA expression of Nrf2

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 14 of 28

and its target genes, NQO1 and HO-1 [190]. The induction of the AMPK-Nrf2-NQO1 signaling pathway has been shown to protect hippocampal cells from oxygen–glucose deprivation-reoxygenation-induced injury [95]. Targeting chromobox 7, a transcriptional repressor, has been shown to influence Nrf2, HO-1, and NQO1 levels as well as behavioral and pathological outcomes in rat ischemic brains in vivo [191].

Experimental studies have also shown that the induction of the Nrf2-ARE pathway in the brain following subarachnoid hemorrhage (SAH) leads to an enhancement of Nrf2-ARE-related factors, including Nrf2, HO-1, and NQO1 [192]. Increased expression of Nrf2-ARE pathway-related agents, such as Nrf2 and NQO1, attenuated SAH-induced early brain injury in rats [193–195]. Geniposide, an anti-inflammatory and anti-oxidative herbal compound, has been shown to reduce brain edema, attenuate blood–brain barrier permeability, inhibit neurocyte apoptosis in the temporal cortex, and improve behavioral function following SAH in rats. These effects were associated with decreased levels of ROS and upregulation of Nrf2 and NQO1 levels [196]. The use of RTA 408, an Nrf2 inducer, can attenuate SAH-induced morphological damages by the enhancement of Nrf2, HO-1, and NQO1 values in a rodent model of SAH [197]. Another Nrf2 inducer, tert-butylhydroquinone, upregulated the expression of KEAP1, Nrf2, HO-1, and NQO1, and ameliorated brain edema, blood–brain barrier dysfunction, behavioral impairment, and cell injury/death after SAH in a rat in vivo model [198]. Propofol, a widely used intravenous anesthetic with sedative and hypnotic properties, has been shown to have neuroprotective effects after ischemic insults and traumatic brain injury (TBI) [199]. It has been demonstrated that propofol attenuates SAH early brain injury through the upregulation of the immunoreactivity of p-Akt, Nrf2, and NQO1 in adult rats in vivo [200]. The future of targeting NQO1 as a potential therapeutic intervention needs a deeper understanding of the complex mechanisms regulating the action of the Nrf2-NQO1 signaling pathway in the development of cerebrovascular diseases. With limited clinical data available, there is a need for additional clinical studies to assess the safety and effectiveness of targeting NQO1 for the treatment of cerebrovascular diseases [201]. Investigating the synergistic effects of combined therapies (e.g., NQO1 modulators in combination with traditional/conventional therapies) could provide insights into maximizing therapeutic outcomes.

NQO1 and TBI

Oxidative stress plays a substantial role in mitochondrial dysfunction and activation of excitatory pathways in both acute and late phases of TBI [202]. The Nrf2 pathway exerts a neuroprotective role in TBI by the alleviation of oxidative damage and neuroinflammatory responses [203]. The fluid percussion injury model, a commonly used experimental approach, provides valuable insights into TBI research, particularly for clinical TBI without skull fracture [204]. The downregulation of Nrf2 and its downstream factor NQO1 in TBI leads to the activation of proinflammatory mediators, such as TGF-β1 and NF-kB, and exacerbates cell injury and death in a fluid percussion injury mouse model of TBI [205]. The genetic deletion of Nrf2 hampers the recovery of both motor and cognitive functions after TBI [206]. The human brain is a suitable tissue for TBI research if it possesses characteristics suitable for modeling TBI [207]. Investigations conducted on tissues obtained during neurosurgery from 31 patients with TBI have revealed that the activation of the Nrf2 pathway may provide neuroprotection against TBI secondary brain injury. These patients were adults with surgical indications, such as progressive cognitive deficits and neurological impairment with no prior neurological disease. Five other specimens of control brain tissue were obtained from patients with benign tumors. In these traumatic brain specimens, significant increases in the nuclear value and decreases in the cytoplasmic level of Nrf2 were associated with an enhancement of the NQO1 and GST upregulation [208].

Several substances targeting the Nrf2 pathway and its downstream factors exert an appropriate protective effect against the detrimental procedures of TBI in the brain [209]. Sulforaphane, an Nrf2 activator, induces NQO1 activity and improves the function of the neurovascular unit following TBI by reducing the loss of endothelial cells and tight junction proteins and preserving blood–brain barrier integrity [210]. The application of curcumin, an anti-oxidative natural substance, decreased neocortex injury, neutrophil infiltration, and microglia activation, induced the nuclear translocation of Nrf2, and enhanced the expression of downstream targets such as HO-1and NQO1 in mice following TBI [211]. Furthermore, the attenuation of neuroinflammatory response through the administration of dexmedetomidine has been shown to prevent neuronal apoptosis and improve behavioral function after TBI in rats. This was accompanied by the promotion of Toll-like receptor 4 expression and upregulation of HO-1 and NQO1 [212, 213]. The administration of tert-butylhydroquinone, an antioxidant compound, reduced the volume of the hypoxic lesions and improved motor function and cognitive deficits after TBI in a cortical impact model in rats. These beneficial outcomes were associated with a significant increase in the nuclear accumulation of Nrf-2 and expression of HO-1 and NQO1 [214]. Targeting mitochondrial oxidative stress by mitoquinone, a strong mitochondrial-targeted antioxidant,

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 15 of 28

has shown promising results in improving neurological deficits, alleviating brain edema, and inhibiting neuronal apoptosis in a rodent model of TBI. This approach is associated with accelerated Nrf2 nuclear translocation and consequently the upregulation of HO-1 and NQO1 [215]. The administration of erythropoietin significantly enhanced the mRNA expression and activities of Nrf2 and its downstream enzyme NQO1, leading to a significant improvement in secondary TBI brain injury in mice [216]. Administration of Irisin, a hormone-like protein that is produced in response to physical activity, increased expression of NQO1 that may contribute to the neuroprotective effects associated with endurance exercise in TBI [217]. Comprehensive studies of how exercise interventions might be optimized post-TBI to harness NQO1-related neuroprotection have the potential to be groundbreaking. Exploring the modulation of the Nrf2 pathway and its downstream factors for alleviating the detrimental effects of TBI and promoting neuroprotection offers exciting prospects for future studies [209]. Furthermore, the integration of multi-omics data may provide a comprehensive understanding of TBI pathogenesis and recovery, with NQO1 as a potential focal point [218].

NQO1 and epilepsy
Chronic inflammation induces mitochondrial dysfunction by generating ROS, which in turn leads to mutations in mitochondrial DNA. This process contributes to neuronal cell injury and loss in patients with epilepsy, especially individuals with medically intractable epilepsy [219, 220]. The Nrf2 signaling pathway has appeared as a critical mechanism for combating seizure-induced ROS, cell death, and persistent seizures in epilepsy. A notable upregulation of Nrf2 mRNA expression was detected in human brain tissue obtained from patients with epilepsy [221]. A study involving 31 temporal lobe specimens obtained during epilepsy surgery revealed a significant increase in Nrf2 mRNA expression compared to autoptic tissues. Furthermore, using an adeno-associated virus to overexpress human Nrf2, the expression levels of Nrf2 and NQO1 increased progressively in mice, reaching a peak at 72 h after pilocarpine-induced status epilepticus. The mice injected with the virus exhibited significantly fewer generalized seizures, reduced microglia activation, and less damage to hippocampal neurons [221]. NQO1 expression in the human epileptic brain may also be linked to the outcomes of epilepsy surgery. An investigation of the brain specimens from 26 patients who underwent surgery for medically refractory epilepsy revealed a significant reduction of NQO1 expression among patients who experienced aura after surgery (ILAE class 2), in comparison to seizure-free patients (ILAE class 1)

[222]. Activation of this pathway has been suggested as a potential interventional approach to alleviating epilepsy and associated comorbidities [223]. To investigate the in vivo functions of NQO1, NQO1-/- mice have been generated [224]. It has been reported that some of the NQO1 (−/−) mice exhibit seizures, which are characterized by tail tension, bending of the front legs, rolling onto their side, salivation, and occasional spasms [113]. Interestingly, the administration of valproic acid, an antiepileptic drug, to the mouse embryo tissues after treatment with the Nrf2 antioxidant pathway inducer, 1,2-dithiole-3-thione, significantly increased the expression of NQO1 [225]. These findings indicate the implication of NQO1 in the modulation of brain excitability.

The expressions of nuclear Nrf2, HO-1, and NQO1 were upregulated 24 h after the onset of lithium chloride-pilocarpine-induced status epilepticus in sixty male Sprague–Dawley rats. However, the extent of this upregulation was insufficient to effectively counteract oxidative stress damage, attenuate lipid peroxidation, and reduce apoptosis [226]. Enhancement of nuclear Nrf2, NQO1, and HO-1 by the application of hydroxylated fullerenes, known as a potent free radical scavenger, significantly reduced lipid peroxidation and apoptosis in the hippocampus of rats following status epilepticus [226]. The administration of dimethyl fumarate, an activator of the Nrf2 pathway, significantly reduced seizures, suppressed pro-inflammatory cytokines, and enhanced the gene expression of Nrf2, NQO1, and HO-1 in the chemical kindling epilepsy rat model [227]. Comparable outcomes have been observed in fifty male Wistar rats subjected to amygdala kindling [89]. Nrf2 expression levels, along with the three Nrf2-regulated genes HO-1, NQO1, and GST, exhibited a notable increase in mice with status epilepticus. The overexpression of human Nrf2 using an adeno-associated virus vector led to a marked decrease in generalized seizures as well as a reduction in microglia activation and neuronal injury in these mice [228]. In a study on 80 male Sprague–Dawley rats, genistein, a naturally occurring isoflavone substance, counteracted the reduction of Nrf2, HO-1, and NQO1 levels following recurrent seizures induced by pentylenetetrazol. This effect is accompanied by a prolongation of seizure latency and a reduction in seizure intensity and duration of generalized seizures. Moreover, this substance exhibits a protective effect on epilepsy-induced brain damage [229]. A total of 60 adult male Wistar rats were used to evaluate the antiepileptic and neuroprotective effects of dehydroepiandrosterone, a steroid hormone, against iron-induced epilepsy [230]. The iron-induced epileptiform burst discharges in rats simulate post-traumatic epilepsy in humans [231]. Treatment with dehydroepiandrosterone, a steroid hormone, has also demonstrated

the ability to suppress oxidative stress, reduce neuronal apoptosis, and improve electrophysiological alterations in an iron-induced epilepsy model in rats. This alleviative effect was associated with the upregulation of Nrf2 and NQO1 [230].

NQO1 and ALS

The main pathological mechanisms of ALS include oxidative stress, neuroinflammation, and mitochondrial dysfunction [232, 233]. Several experimental in vitro and in vivo studies as well as clinical trials revealed that the KEAP1-Nrf2 pathway as well as its downstream mediators has been recognized as key players in the evolution of ALS. Perturbation in the Nrf2/ARE signaling pathway, which is characterized by alterations in Nrf2 expression, may contribute to the chronic degeneration of motor neurons in ALS [234]. In postmortem brain and lumbar spinal cord tissues obtained from 5 patients with ALS, a reduction in both Nrf2 mRNA and protein expression levels has been observed in neurons within the motor cortex and spinal cord compared to autoptic specimens from 5 individuals without any neurological or psychiatric disease, suggesting an impaired capacity of these cells to counteract oxidative stress. These alterations were associated with an enhancement of KEAP1 mRNA expression, particularly in the motor cortex [235]. The activation of the Nrf2/ARE pathway in a transgenic familial ALS mice model demonstrated significant clinical benefits, including reduced weight decline, improved motor impairments, and prolonged survival. Furthermore, this effect was assessed in NSC-34 SOD1-G93A cells, a specialized cell line derived from motor neuron-like cells that have been transfected to express a mutant form of the human superoxide dismutase 1 (SOD1) gene. The activation of the Nrf2 pathway in these cells resulted in a significant upregulation of Nrf2-regulated genes, including NQO1, GST, and HO-1 [236]. Furthermore, in vitro exposure of astrocytes isolated from the spinal cord of symptomatic SOD1-G93A mice and human astrocytes to mesenchymal stem cell-derived extracellular vesicles reduced their neurotoxicity towards motor neurons, potentially mediated by miRNAs carried by the extracellular vesicles. Transfecting human astrocytes with miR-29b-3p leads to the upregulation of NQO1 antioxidant activity and a reduction in neurotoxicity towards motor neurons. This study suggests the therapeutic potential of extracellular vesicles in various subtypes of ALS [237]. It has been reported that familial forms of ALS are accompanied by mutations in the SOD1 gene. The elimination of the Nrf2 gene in SOD1-G93A mice exerted a significant effect on NQO1 expression among the other recognized Nrf2-regulated enzymes. Knocking out Nrf2 in these transgenic mice resulted in accelerated motor

neuron loss and astrocytic proliferation, and led to earlier disease onset and shorter lifespan [238]. Investigation on the lymphoblasts of 10 patients (six sporadic ALS and four male SOD1-ALS patients) has shown an increase in Nrf2, HO-1, and NQO1 protein values in patients with sporadic ALS. However, in lymphoblasts carrying mutations in the SOD1 gene, only a significant decrease in the NQO1 mRNA levels has been observed. This suggests a potential disruption in the transcriptional regulation of NQO1 in the presence of mutated SOD1 in ALS [239]. An investigation on lymphoblasts derived from 10 patients with ALS (six sporadic ALS and four SOD1-ALS patients) revealed that the baseline levels of the Nrf2 in lymphoblasts derived from patients with SOD1-related ALS were comparable to those of control cells derived from seven healthy subjects. Treatment with combined structural fragments fasudil and the Nrf2 inductors/radical scavengers ferulic and caffeic acids led to a significant increase in NQO1 levels at both the mRNA and protein values [240]. These findings point to the potential therapeutic efficacy of targeting NQO1 in ALS subjects with mutations in the SOD1 gene.

The implication of ATP-gated P2X7 ion channel in the progression of ALS has been identified [241]. In a study involving 47 SOD1-G93A mice, the inhibition of the ATP-gated P2X7 ion channel by the CNS-penetrant P2X7 antagonist JNJ-47965567 resulted in a significant reduction in NQO1 gene expression [242]. A cell culture study revealed that mutations in TANK-binding kinase 1 and sequestosome 1, which are genetic risk factors for ALS, disrupted selective autophagy in association with a dysregulation in the levels of NQO1 and KEAP1 [243]. Pathological transactive response DNA-binding protein 43 (TDP-43) is also involved in the pathogenesis of ALS [244]. In an ALS cell model featuring human mutant TDP-43 within the NSC-34 cell line, the levels of total and cytoplasmic Nrf2, as well as its downstream gene NQO1, significantly decreased in a mutant TDP-43 transgenic cell [245]. Treatment of motor neuron-like NSC34 cells overexpressing TDP-43 with diallyl trisulfide, an organosulfur compound in garlic oil with anti-inflammatory properties, activates the Nrf2/ARE pathway, upregulating HO-1 and NQO1 expression, and protects cells from TDP-43-induced damage by scavenging ROS [246]. Sulforaphane enhances the expression of HO-1 and NQO1 in motor neuron-like cells transfected with the wild-type TDP-43 [247]. To validate the significance of NQO1 in the treatment of ALS, comprehensive human studies are essential. These studies may involve retrospective analyses of existing patient data and implementation of prospective clinical trials to evaluate potential therapeutic approaches.

NQO1 and brain tumors

ROS-sensitive cell signaling pathways play a vital role in the survival, proliferation, and differentiation of tumor cells in various types of brain cancers. These pathways significantly modulate the metabolism, inflammatory processes, and angiogenesis accompanied by brain tumors [248]. Investigations conducted on human glioblastoma (GBM) involving ten patients, as well as primary brain tissue cell cultures and human neuroblastoma (SH-SY5Y) cells, have revealed that the regulation of the Nrf2 pathway and the expression of downstream antioxidant enzymes, such as NQO1, HO-1, and GST, are crucial in the regulation of ROS activity, detoxification of xenobiotics, and inhibition of brain cancer progression [50, 249, 250]. NQO1 contributes to various dimensions of tumorigenesis and treatment response, particularly via the regulation of inflammatory processes. The effect of the ROS retractor C/EBPβ on NQO1 expression in GBM has been evaluated in human glioblastoma cell lines and within an in vivo xenograft animal model. It has been reported that C/EBPβ can counteract ROS in GBM by activating NQO1. This activation of NQO1 exerted an anti-oxidative stress response and reduced the detrimental effects of ROS on GBM cells [44]. Under physiological states, the nuclear-restricted protein (NRP/B) in the brain is typically localized within the nucleus. NRP/B together with Nrf2 plays a crucial role in the protection of human GBM cells against oxidative stress-induced damage [50]. When exposed to oxidative stress, Nrf2 and NQO1 work together to prevent the detrimental effects of oxidative stress. However, in primary brain tumors, NRP/B relocates from the nucleus to the cytoplasm. This cytoplasmic localization of NRP/B alters the function of Nrf2, consequently affecting the transcription and function of downstream genes, including NQO1. The relocation of NRP/B and the alteration of Nrf2-NQO1 function diminish the protective effect against oxidative stress, which is potentially contributed to brain tumorigenesis [50, 249, 251].

NQO1 plays a dual and contradictory role as an anticancer enzyme and an oncogene in the evolution of GBM. Overexpressing NQO1 significantly enhances the cell growth of U87MG and LN229 GBM cells. Conversely, depletion of NQO1 significantly reduces cell proliferation, suggesting the critical role of NQO1 values in determining the proliferation of these tumor cells [252]. NQO1, acting as a downstream target gene of phosphatase and tensin homolog, can effectively reduce ROS values in GBM and facilitate tumorigenesis [252]. Relevant studies demonstrated that the inactivation of glutathione-S-transferase Pi 1 (GSTP1) and NQO1 amplifies ROS-induced tissue damage and leads to the induction of apoptosis and consequently to the inhibition of U87MG GBM cell proliferation. Thus, inhibition of GSTP1 and NQO1 is suggested as a new treatment strategy for GBM [253]. Furthermore, the expression levels of NQO1 can impact the efficacy of tumor treatment in specific types of brain tumors. A correlation has been described between high levels of NQO1 expression and increased radiation resistance in primary mouse models of diffuse intrinsic pontine glioma. The intrinsic radiosensitivity of brainstem gliomas was governed by the p53 signaling pathway, which was linked to the suppression of the Nrf2 pathway gene *NQO1*. The deletion of p53 led to the upregulation of NQO1 expression and a substantial enhancement in tumor resistance to radiation therapy in vivo. The modulation of the elevated levels of NQO1 in tumors suggests a potential method to enhance the efficacy of radiotherapy in brainstem gliomas [254]. NQO1 expression value has the potential to serve as a prognostic biomarker in GBM. Using a dataset with 31 samples derived from the Cancer Genome Atlas database, a positive correlation has been revealed between NQO1 expression and the degree of malignancy in GBM tissues. Modulating NQO1 levels can potentially induce anticancer effects through a non-apoptotic necrosis mechanism in U87MG and U251 glioma cells [255]. NQO1, an antioxidant factor and a protector of p53, plays a crucial role in protecting cells against oxidative stress and DNA damage in head and neck tumors [256]. Alterations in NQO1 function and/or expression can disrupt the equilibrium of cell death pathways, potentially resulting in NQO1-dependent necrosis as a cellular demise mechanism [3, 257, 258]. The administration of temozolomide, an alkylating agent commonly applied for the treatment of GBM, can interact with NQO1 and lead to non-apoptotic necrosis-induced cell death in human U251MG and U87MG GBM cells [259]. Various other potential antitumor compounds, including 2-methoxy-6-acetyl-7-methyljuglone [255], chlorpyrifos (an organophosphate pesticide) [260], and FTY720 (a sphingosine-1-phosphate receptor modulator) [259], have been found to exert cytotoxic effects on various GBM cancer cells by targeting NQO1. Multiple studies have demonstrated that individuals with glioma carrying the isocitrate dehydrogenase 1 (IDH1) R132H mutation show greater sensitivity to the treatment with temozolomide [261]. The IDH1 mutation can lead to a decrease in NADPH values and the accumulation of ROS in GBM cells. Interestingly, when IDH1 R132H cells were exposed to temozolomide, a reduction in NQO1 expression has been observed. This observation suggests a potential involvement of NQO1 in the resistance mechanism of GBM cells to chemotherapy [262]. Studies on fourteen primary and recurrent GBM specimens obtained from patients receiving temozolomide and radiation therapy demonstrated a correlation between the nuclear Nrf2

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 18 of 28

levels and the time to GBM recurrence. The combined treatment of temozolomide and ionizing radiation has been shown to activate the Nrf2-ARE pathway in human GBM cells. This activation leads to a significant increase in the expression of NQO1 and HO-1, which suggests their involvement in the cellular response to this treatment regimen [263]. Investigations on four different GBM cell lines have shown that the diminished activity of NQO1 is a possible mechanism of resistance to heat shock protein 90 inhibitors in GBM [59]. Pharmacological chaperones offer promising prospects for rectifying the misfolding of NQO1 and enhancing enzyme stability and function. This approach could play a therapeutic role in diverse neurological diseases, including brain tumors [72]. The utilization of a small molecular chaperone exhibited the ability to restore the native wild-type conformation of NQO1 and a substantial enhancement in the enzymatic activity of the P187S variant protein [264]. Furthermore, by using four glioma cell lines (U251, T98G, LN-229, and A172), it has been shown that the interplay between NQO1 and the anti-oncogenic miR-1321 with the oncogene serpin family A member 1 has been shown to impact the proliferation and apoptosis of glioma cells [265]. Targeting the NQO1-mediated pathway of ferroptosis represents another novel therapeutic strategy for the treatment of glioma [266]. Reducing ferroptosis by suppressing KEAP1, which leads to the nuclear translocation of Nrf2 and enhanced expression of NQO1 and HO-1, has demonstrated significant inhibition of U87 GBM cell xenograft growth in the brain ventricles of nude mice [267]. Moreover, NQO1 shows potential as a diagnostic marker for different types of tumors. Theranostic prodrugs based on NQO1 allow for the monitoring of quinone moiety reduction and release of the parent drug, while also showing selective cytotoxicity against cancer cells [268]. Future studies on the role of NQO1 in the pathogenesis of brain tumors can investigate the interactions of NQO1 with various brain tumorigenesis pathways, personalized therapies based on NQO1 levels and genetic profiles, feedback mechanisms regulating NQO1 in brain tumors, and the factors triggering the transition of NQO1 from a protective to a detrimental role in GBM.

Developing novel therapeutic and/or diagnostic compounds by targeting NQO1

In the past few years, significant progress has been made in uncovering the pathological roles of NQO1 in various neurological disorders. Exogenous compounds that act as modulators of NQO1 have been extensively studied. However, the identification of endogenous substances that modulate this enzyme necessitates further in-depth investigations. The different roles of NQO1 in the CNS present a significant challenge in the selective modulation of a single isolated function by novel compounds [3]. Various substances that have been developed to regulate the NQO1 enzyme could also interact with other enzymes or proteins, such as cytochrome P450 reductase [269]. Efforts are underway to design compounds that selectively target NQO1 without interacting with other crucial cellular processes or proteins and minimize off-target effects [270, 271]. Integrating a feedback mechanism or regulating enzyme expression may reduce potential off-target effects. Considering the functions of NQO1 in reducing oxidative stress through various mechanisms, such as acting as a redox switch and influencing cellular damage and death by generating NAD^+, it is valuable to consider further efforts in the development of novel compounds to fight against neuroinflammation, neurodegeneration, and the progression of brain malignancies. Nanotechnology has emerged as a novel approach to enhance the delivery of bioactivatable substances targeting NQO1 to specific cell types [272]. Using nano-carriers, such as nano-particles, liposomes, or amphiphilic polymers, can promote the stability, solubility, bioavailability, and ability to penetrate the blood–brain barrier of NQO1-targeted drugs [273]. One of the major challenges in using NQO1-responsive pro-drugs and nano-carriers is the possibility of unintended activation by other reductase enzymes, such as P450 reductase and NADPH [274]. The cross-reactivity of these enzymes can result in off-target effects and compromise the specificity of drug delivery. Cutting-edge techniques, such as the enzyme-responsive nano-drug delivery system, offer a valuable approach for fabricating nano-carriers that exhibit precise targeting and reductive release [275]. The integration of multiple activities within a single molecular entity has been used to design stable and potent peptide inhibitors of the Nrf2-KEAP1 pathway with optimal cellular uptake and resistance against degradation in human serum [276]. Investigating the long-term stability and biodegradability of the nano-carriers would be essential from both environmental and systemic health standpoints [277]. Furthermore, due to selectivity, potency, pharmacokinetic properties, and the ready availability of diagnostics for assessing NQO1 in patients, some NQO1 substrates such as deoxynyboquinone, have considerable potential as personalized medicines for the treatment of various disorders, such as brain tumors [278].

Using the enzymatic characteristics of NQO1, a variety of NQO1-activated optical probes for imaging different cells in various tissues, including the brain, have been developed [279]. This capability could be used for detecting early alterations in neuronal tissues and serve as a promising diagnostic tool in neurological disorders [274, 280]. Indeed, fluorescent probes specifically designed

for cellular and intra-vital imaging of mitochondrial NQO1 have shown remarkable binding affinity to NQO1. These probes have been successfully used to differentiate between different NQO1-expressing cancer cells and normal cells [274]. Moreover, they have provided valuable insights into the decreased NQO1 activity observed in a cellular model of PD [274]. Fluorescent probes have also been developed to enable non-invasive monitoring of endogenous NQO1 activity in brain tumor cells both in vitro and in transplanted tumors in nude mice [281]. An NQO-1-activated near-infrared agent has been designed to rich into mitochondria and lysosomes and act as a chemotherapeutic compound [282]. A genetically encoded sensor-based metabolic screening tool has been also developed for tracking intracellular NQO1-activated redox cycling [283].

Conclusion

NQO1 plays a regulatory role in the oxidant/antioxidant equilibrium within the brain by facilitating the detoxification of harmful compounds, protecting cells from oxidative stress, regenerating antioxidants, and its ability to directly interact with other molecules [3, 269, 284]. Available data strongly suggest that targeting NQO1 could be a promising strategy for developing new diagnostic and therapeutic compounds for neurological disorders. Furthermore, NQO1 can be utilized as a biomarker for tracking subtle changes in neural tissues and serve as an early warning system for the emergence of neurological diseases. To develop suitable compounds for targeting NQO1, additional experimental and clinical studies are required to improve our understanding of the basic molecular mechanisms of NQO1 and its particular role in the evolution and progression of neurological disorders. When considering the potential contributions of NQO1 to personalized medicine for neurological disorders, evaluation of its potential interactions with other neurological biomarkers, and using artificial intelligence and big data for a deeper understanding of the complex role of NQO1 in neurological disorders may open up exciting research paths for the future. Acknowledging potential off-target effects, specific conditions where NQO1 may not be a reliable biomarker, and the challenges of translating laboratory discoveries to clinical practice provide a well-rounded and balanced perspective on the role of NQO1 in the field of personalized medicine. Furthermore, the significance lies in the adoption of replicable research designs and methodologies across diverse populations. It is important to note that potential off-target effects, specific conditions in which NQO1 may

not function as a reliable biomarker, and the difficulties in translating bench side findings to the bedside represent significant challenges for future studies. Furthermore, the potential different roles of NQO1 in acute and chronic neurological disorders should be considered in future studies.

Abbreviations

ATP	Adenosine triphosphate
AD	Alzheimer´s disease
ALS	Amyotrophic lateral sclerosis
Aβ	Amyloid-β
AMPK	AMP-activated protein kinase
AP-1	Activator protein 1
ARE	Antioxidant response element enhancer sequence
BDNF	Brain-derived neurotrophic
CNS	Central nervous system
CREB	CAMP response element-binding protein
DA	Dopamine
EAE	Experimental autoimmune encephalomyelitis mouse model
FAD	Flavin adenine dinucleotide
GST	Glutathione S-transferase
GBM	Glioblastoma
GSTP1	Glutathione-S-transferase Pi 1
HIF-1α	Hypoxia-inducible factor 1-alpha
HO-1	Heme oxygenase-1
IDH1	Isocitrate dehydrogenase 1
KEAP1	Kelch-like ECH-associated protein1
MCAO	Middle cerebral artery occlusion
MS	Multiple sclerosis
NQO1	NAD(P)H Quinone Dehydrogenase 1
Nrf2	Nuclear factor erythroid 2-related factor 2
NRP/B	Nuclear-restricted protein
PD	Parkinson's disease
PI3K	Phosphoinositide 3-kinase
RIPK1	Receptor-interacting serine/threonine-protein kinase 1
ROS	Oxidative stresses
SAH	Subarachnoid hemorrhage
SN	Substantia nigra
SOD1	Superoxide dismutase 1
TBI	Traumatic brain injury
TDP-43	Transactive response DNA-binding protein 43

Availability of data and materials
The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 20 of 28

References

1. Siegel D, Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. Free Radical Biol Med. 2000;29(3–4):246–53. https://doi.org/10.1016/s0891-5849(00)00310-5.
2. Janda E, Nepveu F, Calamini B, Ferry G, Boutin JA. Molecular Pharmacology of NRH: Quinone Oxidoreductase 2: a Detoxifying Enzyme Acting as an Undercover Toxifying Enzyme. Mol Pharmacol. 2020;98(5):620–33. https://doi.org/10.1124/molpharm.120.000105.
3. Ross D, Siegel D. The diverse functionality of NQO1 and its roles in redox control. Redox Biol. 2021;41: 101950. https://doi.org/10.1016/j.redox.2021.101950.
4. Xiao FY, Jiang ZP, Yuan F, Zhou FJ, Kuang W, Zhou G, Chen XP, Liu R, Zhou HH, Zhao XL, Cao S. Down-regulating NQO1 promotes cellular proliferation in K562 cells via elevating DNA synthesis. Life Sci. 2020;248: 117467. https://doi.org/10.1016/j.lfs.2020.117467.
5. Oh ET, Kim HG, Kim CH, Lee J, Kim C, Lee JS, Cho Y, Park HJ. NQO1 regulates cell cycle progression at the G2/M phase. Theranostics. 2023;13(3):873–95. https://doi.org/10.7150/thno.77444.
6. Zhou HZ, Zeng HQ, Yuan D, Ren JH, Cheng ST, Yu HB, Ren F, Wang Q, Qin YP, Huang AL, Chen J. NQO1 potentiates apoptosis evasion and upregulates XIAP via inhibiting proteasome-mediated degradation SIRT6 in hepatocellular carcinoma. Cell Commun Signal. 2019;17(1):168. https://doi.org/10.1186/s12964-019-0491-7.
7. Park JE, Park JS, Leem YH, Kim DY, Kim HS. NQO1 mediates the anti-inflammatory effects of nootkatone in lipopolysaccharide-induced neuroinflammation by modulating the AMPK signaling pathway. Free Radical Biol Med. 2021;164:354–68. https://doi.org/10.1016/j.freeradbiomed.2021.01.015.
8. Li R, Zhou Y, Zhang S, Li J, Zheng Y, Fan X. The natural (poly)phenols as modulators of microglia polarization via TLR4/NF-κB pathway exert anti-inflammatory activity in ischemic stroke. Eur J Pharmacol. 2022;914: 174660. https://doi.org/10.1016/j.ejphar.2021.174660.
9. Luo J, Li S, Qin X, Peng Q, Liu Y, Yang S, Qin X, Xiong Y, Zeng Z. Association of the NQO1 C609T polymorphism with Alzheimer's disease in Chinese populations: a meta-analysis. Int J Neurosci. 2016;126(3):199–204. https://doi.org/10.3109/00207454.2015.1004573.
10. Alexoudi A, Zachaki S, Stavropoulou C, Chatzi I, Koumbi D, Stavropoulou K, Kollia P, Karageorgiou CE, Sambani C. Combined GSTP1 and NQO1 germline polymorphisms in the susceptibility to Multiple Sclerosis. Int J Neurosci. 2015;125(1):32–7. https://doi.org/10.3109/00207454.2014.899597.
11. Osama A, Zhang J, Yao J, Yao X, Fang J. Nrf2: a dark horse in Alzheimer's disease treatment. Ageing Res Rev. 2020;64: 101206. https://doi.org/10.1016/j.arr.2020.101206.
12. Riley RJ, Workman P. DT-diaphorase and cancer chemotherapy. Biochem Pharmacol. 1992;43(8):1657–69. https://doi.org/10.1016/0006-2952(92)90694-e.
13. Talalay P, Fahey JW, Holtzclaw WD, Prestera T, Zhang Y. Chemoprotection against cancer by phase 2 enzyme induction. Toxicol Lett. 1995;82–83:173–9. https://doi.org/10.1016/0378-4274(95)03553-2.
14. Jaiswal AK. Human NAD(P)H:quinone oxidoreductase (NQO1) gene structure and induction by dioxin. Biochemistry. 1991;30(44):10647–53. https://doi.org/10.1021/bi00108a007.
15. Zhong X, Ma Z, Su Y, Li Z, Liao Y, Pan X, Zang L, Zhou S. Flavin adenine dinucleotide ameliorates hypertensive vascular remodeling via activating short chain acyl-CoA dehydrogenase. Life Sci. 2020;258: 118156. https://doi.org/10.1016/j.lfs.2020.118156.
16. Li R, Bianchet MA, Talalay P, Amzel LM. The three-dimensional structure of NAD(P)H:quinone reductase, a flavoprotein involved in cancer chemoprotection and chemotherapy: mechanism of the two-electron reduction. Proc Natl Acad Sci USA. 1995;92(19):8846–50. https://doi.org/10.1073/pnas.92.19.8846.
17. Pey AL, Megarity CF, Medina-Carmona E, Timson DJ. Natural small molecules as stabilizers and activators of cancer-associated NQO1 polymorphisms. Curr Drug Targets. 2016;17(13):1506–14. https://doi.org/10.2174/1389450117666160101121610.
18. Bianchet MA, Faig M, Amzel LM. Structure and mechanism of NAD[P]H:quinone acceptor oxidoreductases (NQO). Methods Enzymol. 2004;382:144–74. https://doi.org/10.1016/S0076-6879(04)82009-3.
19. Siegel D, Kepa JK, Ross D. NAD(P)H:quinone oxidoreductase 1 (NQO1) localizes to the mitotic spindle in human cells. PLoS ONE. 2012;7(9): e44861. https://doi.org/10.1371/journal.pone.0044861.
20. Lind C, Cadenas E, Hochstein P, Ernster L. DT-diaphorase: purification, properties, and function. Methods Enzymol. 1990;186:287–301. https://doi.org/10.1016/0076-6879(90)86122-c.
21. Doughan AK, Dikalov SI. Mitochondrial redox cycling of mitoquinone leads to superoxide production and cellular apoptosis. Antioxid Redox Signal. 2007;9(11):1825–36. https://doi.org/10.1089/ars.2007.1693.
22. Siegel D, Gustafson DL, Dehn DL, Han JY, Boonchoong P, Berliner LJ, Ross D. NAD(P)H:quinone oxidoreductase 1: role as a superoxide scavenger. Mol Pharmacol. 2004;65(5):1238–47. https://doi.org/10.1124/mol.65.5.1238.
23. Winski SL, Koutalos Y, Bentley DL, Ross D. Subcellular localization of NAD(P)H:quinone oxidoreductase 1 in human cancer cells. Can Res. 2002;62(5):1420–4.
24. Schlager JJ, Powis G. Cytosolic NAD(P)H:(quinone-acceptor)oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. Int J Cancer. 1990;45(3):403–9. https://doi.org/10.1002/ijc.2910450304.
25. Peng Q, Lu Y, Lao X, Chen Z, Li R, Sui J, Qin X, Li S. The NQO1 Pro187Ser polymorphism and breast cancer susceptibility: evidence from an updated meta-analysis. Diagn Pathol. 2014;9:100. https://doi.org/10.1186/1746-1596-9-100.
26. van Horssen J, Schreibelt G, Bö L, Montagne L, Drukarch B, van Muiswinkel FL, de Vries HE. NAD(P)H:quinone oxidoreductase 1 expression in multiple sclerosis lesions. Free Radical Biol Med. 2006;41(2):311–7. https://doi.org/10.1016/j.freeradbiomed.2006.04.013.
27. Vabulas RM. Ferroptosis-related flavoproteins: their function and stability. Int J Mol Sci. 2021;22(1):430. https://doi.org/10.3390/ijms22010430.
28. Preethi S, Arthiga K, Patil AB, Spandana A, Jain V. Review on NAD(P)H dehydrogenase quinone 1 (NQO1) pathway. Mol Biol Rep. 2022;49(9):8907–24. https://doi.org/10.1007/s11033-022-07369-2.
29. Asher G, Tsvetkov P, Kahana C, Shaul Y. A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppressors p53 and p73. Genes Dev. 2005;19(3):316–21. https://doi.org/10.1101/gad.319905.
30. Sun C, Zhao W, Wang X, Sun Y, Chen X. A pharmacological review of dicoumarol: an old natural anticoagulant agent. Pharmacol Res. 2020;160: 105193. https://doi.org/10.1016/j.phrs.2020.105193.
31. Beyer RE, Segura-Aguilar J, Di Bernardo S, Cavazzoni M, Fato R, Fiorentini D, Galli MC, Setti M, Landi L, Lenaz G. The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. Proc Natl Acad Sci USA. 1996;93(6):2528–32. https://doi.org/10.1073/pnas.93.6.2528.
32. Asher G, Lotem J, Kama R, Sachs L, Shaul Y. NQO1 stabilizes p53 through a distinct pathway. Proc Natl Acad Sci USA. 2002;99(5):3099–104. https://doi.org/10.1073/pnas.052706799.
33. Chapple SJ, Cheng X, Mann GE. Effects of 4-hydroxynonenal on vascular endothelial and smooth muscle cell redox signaling and function in health and disease. Redox Biol. 2013;1(1):319–31. https://doi.org/10.1016/j.numecd.2009.12.008.
34. Yu J, Zhong B, Zhao L, Hou Y, Wang X, Chen X. Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) inhibitors Necrostatin-1 (Nec-1) and 7-Cl-O-Nec-1 (Nec-1s) are potent inhibitors of NAD(P)H: Quinone oxidoreductase 1 (NQO1). Free Radical Biol Med. 2021;173:64–9. https://doi.org/10.1016/j.freeradbiomed.2021.07.017.
35. Kim DH, Kundu JK, Surh YJ. Redox modulation of p53: mechanisms and functional significance. Mol Carcinog. 2011;50(4):222–34. https://doi.org/10.1002/mc.20709.
36. Olovnikov IA, Kravchenko JE, Chumakov PM. Homeostatic functions of the p53 tumor suppressor: regulation of energy metabolism and antioxidant defense. Semin Cancer Biol. 2009;19(1):32–41. https://doi.org/10.1016/j.semcancer.2008.11.005.

Yuhan *et al. Journal of Translational Medicine* (2024) 22:4

Page 21 of 28

37. Anwar A, Dehn D, Siegel D, Kepa JK, Tang LJ, Pietenpol JA, Ross D. Interaction of human NAD(P)H:quinone oxidoreductase 1 (NQO1) with the tumor suppressor protein p53 in cells and cell-free systems. J Biol Chem. 2003;278(12):10368–73. https://doi.org/10.1074/jbc.M2119 81200.

38. Wakabayashi N, Slocum SL, Skoko JJ, Shin S, Kensler TW. When NRF2 talks, who's listening? Antioxid Redox Signal. 2010;13(11):1649–63. https://doi.org/10.1089/ars.2010.3216.

39. Oh ET, Park HJ. Implications of NQO1 in cancer therapy. BMB Rep. 2015;48(11):609–17. https://doi.org/10.5483/bmbrep.2015.48.11.190.

40. Garate M, Wong RP, Campos EI, Wang Y, Li G. NAD(P)H quinone oxidoreductase 1 inhibits the proteasomal degradation of the tumour suppressor p33(ING1b). EMBO Rep. 2008;9(6):576–81. https://doi.org/10.1038/embor.2008.48.

41. van Muiswinkel FL, Kuiperij HB. The Nrf2-ARE Signalling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. Current Drug Targ CNS and Neurol Disorders. 2005;4(3):267–81. https://doi.org/10.2174/1568007054038238.

42. Kim HJ, Oh GS, Shen A, Lee SB, Choe SK, Kwon KB, Lee S, Seo KS, Kwak TH, Park R, So HS. Augmentation of NAD(+) by NQO1 attenuates cisplatin-mediated hearing impairment. Cell Death Dis. 2014;5(6): e1292. https://doi.org/10.1038/cddis.2014.255.

43. Meng C, Zhan J, Chen D, Shao G, Zhang H, Gu W, Luo J. The deubiquitinase USP11 regulates cell proliferation and ferroptotic cell death via stabilization of NRF2 USP11 deubiquitinates and stabilizes NRF2. Oncogene. 2021;40(9):1706–20. https://doi.org/10.1038/s41388-021-01660-5.

44. Lei K, Xia Y, Wang XC, Ahn EH, Jin L, Ye K. C/EBPβ mediates NQO1 and GSTP1 anti-oxidative reductases expression in glioblastoma, promoting brain tumor proliferation. Redox Biol. 2020;34: 101578. https://doi.org/10.1016/j.redox.2020.101578.

45. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. Med Res Rev. 2012;32(4):687–726. https://doi.org/10.1002/med.21257.

46. Adibhatla RM, Hatcher JF. Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2010;12(1):125–69. https://doi.org/10.1089/ars.2009.2668.

47. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med. 2009;7:97. https://doi.org/10.1186/1479-5876-7-97.

48. Iadecola C, Anrather J. Stroke research at a crossroad: asking the brain for directions. Nat Neurosci. 2011;14(11):1363–8. https://doi.org/10.1038/nn.2953.

49. Ding Y, Chen M, Wang M, Wang M, Zhang T, Park J, Zhu Y, Guo C, Jia Y, Li Y, Wen A. Neuroprotection by acetyl-11-keto-β-Boswellic acid, in ischemic brain injury involves the Nrf2/HO-1 defense pathway. Sci Rep. 2014;4:7002. https://doi.org/10.1038/srep07002.

50. Seng S, Avraham HK, Birrane G, Jiang S, Li H, Katz G, Bass CE, Zagozdzon R, Avraham S. NRP/B mutations impair Nrf2-dependent NQO1 induction in human primary brain tumors. Oncogene. 2009;28(3):378–89. https://doi.org/10.1038/onc.2008.396.

51. Di Francesco A, Di Germanio C, Panda AC, Huynh P, Peaden R, Navas-Enamorado I, Bastian P, Lehrmann E, Diaz-Ruiz A, Ross D, Siegel D, Martindale JL, Bernier M, Gorospe M, Abdelmohsen K, de Cabo R. Novel RNA-binding activity of NQO1 promotes SERPINA1 mRNA translation. Free Radical Biol Med. 2016;99:225–33. https://doi.org/10.1016/j.freeradbiomed.2016.08.005.

52. Sudheesh AP, Mohan N, Francis N, Laishram RS, Anderson RA. Star-PAP controlled alternative polyadenylation coupled poly(A) tail length regulates protein expression in hypertrophic heart. Nucleic Acids Res. 2019;47(20):10771–87. https://doi.org/10.1093/nar/gkz875.

53. Adamovich Y, Shlomai A, Tsvetkov P, Umansky KB, Reuven N, Estall JL, Spiegelman BM, Shaul Y. The protein level of PGC-1α, a key metabolic regulator, is controlled by NADH-NQO1. Mol Cell Biol. 2013;33(13):2603–13. https://doi.org/10.1128/MCB.01672-12.

54. Ross D, Siegel D. Functions of NQO1 in cellular protection and CoQ10 metabolism and its potential role as a redox sensitive molecular switch. Front Physiol. 2017;8:595. https://doi.org/10.3389/fphys.2017.00595.

55. Mekiten O, Yitzhaky A, Gould N, Rosenblum K, Hertzberg L. Ribosome subunits are upregulated in brain samples of a subgroup of individuals with schizophrenia: a systematic gene expression meta-analysis. J Psychiatric Res. 2023;164:372–81. https://doi.org/10.1016/j.jpsychires.2023.06.013.

56. Iida A, Sekine A, Saito S, Kitamura Y, Kitamoto T, Osawa S, Mishima C, Nakamura Y. Catalog of 320 single nucleotide polymorphisms (SNPs) in 20 quinone oxidoreductase and sulfotransferase genes. J Hum Genet. 2001;46(4):225–40. https://doi.org/10.1007/s100380170093.

57. Zhao P, Zhao L, Zou P, Lu A, Liu N, Yan W, Kang C, Fu Z, You Y, Jiang T. Genetic oxidative stress variants and glioma risk in a Chinese population: a hospital-based case-control study. BMC Cancer. 2012;12:617. https://doi.org/10.1186/1471-2407-12-617.

58. Medina-Carmona E, Betancor-Fernández I, Santos J, Mesa-Torres N, Grottelli S, Batlle C, Naganathan AN, Oppici E, Cellini B, Ventura S, Salido E, Pey AL. Insight into the specificity and severity of pathogenic mechanisms associated with missense mutations through experimental and structural perturbation analyses. Hum Mol Genet. 2019;28(1):1–15. https://doi.org/10.1093/hmg/ddy323.

59. Gaspar N, Sharp SY, Pacey S, Jones C, Walton M, Vassal G, Eccles S, Pearson A, Workman P. Acquired resistance to 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) in glioblastoma cells. Can Res. 2009;69(5):1966–75. https://doi.org/10.1158/0008-5472.CAN-08-3131.

60. Lajin B, Alachkar A. The NQO1 polymorphism C609T (Pro187Ser) and cancer susceptibility: a comprehensive meta-analysis. Br J Cancer. 2013;109(5):1325–37. https://doi.org/10.1038/bjc.2013.357.

61. Chen H, Lum A, Seifried A, Wilkens LR, Le Marchand L. Association of the NAD(P)H:quinone oxidoreductase 609C→T polymorphism with a decreased lung cancer risk. Can Res. 1999;59(13):3045–8.

62. Lin P, Wang HJ, Lee H, Lee HS, Wang SL, Hsueh YM, Tsai KJ, Chen CY. NAD(P)H: quinone oxidoreductase polymorphism and lung cancer in Taiwan. J Toxicol Environ Health A. 1999;58(4):187–97. https://doi.org/10.1080/009841099157287.

63. Song SY, Jeong SY, Park HJ, Park SI, Kim DK, Kim YH, Shin SS, Lee SW, Ahn SD, Kim JH, Lee JS, Choi EK. Clinical significance of NQO1 C609T polymorphisms after postoperative radiation therapy in completely resected non-small cell lung cancer. Lung cancer (Amsterdam, Netherlands). 2010;68(2):278–82. https://doi.org/10.1016/j.lungcan.2009.06.009.

64. Hamajima N, Matsuo K, Iwata H, Shinoda M, Yamamura Y, Kato T, Hatooka S, Mitsudomi T, Suyama M, Kagami Y, Ogura M, Ando M, Sugimura Y, Tajima K. NAD(P)H: quinone oxidoreductase 1 (NQO1) C609T polymorphism and the risk of eight cancers for Japanese. Int J Clin Oncol. 2002;7(2):103–8. https://doi.org/10.1007/s101470200013.

65. Menzel HJ, Sarmanova J, Soucek P, Berberich R, Grünewald K, Haun M, Kraft HG. Association of NQO1 polymorphism with spontaneous breast cancer in two independent populations. Br J Cancer. 2004;90(10):1989–94. https://doi.org/10.1038/sj.bjc.6601779.

66. Fan Y, Hu D, Feng B, Wang W. The NQO1 C609T polymorphism and hepatocellular carcinoma risk. Tumour Biol. 2014;35(8):7343–50. https://doi.org/10.1007/s13277-014-1712-8.

67. Cho CG, Lee SK, Nam SY, Lee MS, Lee SW, Choi EK, Park HJ, Kim SY. Association of the GSTP1 and NQO1 polymorphisms and head and neck squamous cell carcinoma risk. J Korean Med Sci. 2006;21(6):1075–9. https://doi.org/10.3346/jkms.2006.21.6.1075.

68. Belinsky M, Jaiswal AK. NAD(P)H:quinone oxidoreductase1 (DT-diaphorase) expression in normal and tumor tissues. Cancer Metastasis Rev. 1993;12(2):103–17. https://doi.org/10.1007/BF00689804.

69. Buranrat B, Chau-in S, Prawan A, Puapairoj A, Zeekpudsa P, Kukongviriyapan V. NQO1 expression correlates with cholangiocarcinoma prognosis. Asian Pacific journal of cancer prevention: APJCP. 2012;13(Suppl):131–6.

70. Boroumand M, Pourgholi L, Goodarzynejad H, Ziaee S, Hajhosseini-Talasaz A, Sotoudeh-Anvari M, Mandegary A. NQO1 C609T polymorphism is associated with coronary artery disease in a gender-dependent manner. Cardiovasc Toxicol. 2017;17(1):35–41. https://doi.org/10.1007/s12012-015-9353-8.

71. Zhou H, Wan H, Zhu L, Mi Y. Research on the effects of rs1800566 C/T polymorphism of NAD(P)H quinone oxidoreductase 1 gene on cancer risk involves analysis of 43,736 cancer cases and 56,173 controls. Front Oncol. 2022;12: 980897. https://doi.org/10.3389/fonc.2022.980897.

72. Beaver SK, Mesa-Torres N, Pey AL, Timson DJ. NQO1: a target for the treatment of cancer and neurological diseases, and a model to understand loss of function disease mechanisms. Biochimica et Biophysica

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 22 of 28

Acta Proteins Proteomics. 2019;1867(7–8):663–76. https://doi.org/10.1016/j.bbapap.2019.05.002.

73. Glorieux C, Buc Calderon P. Cancer cell sensitivity to redox-cycling Quinones is influenced by NAD(P)H: Quinone oxidoreductase 1 polymorphism. Antioxidants (Basel, Switzerland). 2019;8(9):369. https://doi.org/10.3390/antiox8090369.

74. Kolesar JM, Dahlberg SE, Marsh S, McLeod HL, Johnson DH, Keller SM, Schiller JH. The NQO1*2/*2 polymorphism is associated with poor overall survival in patients following resection of stages II and IIIa non-small cell lung cancer. Oncol Rep. 2011;25(6):1765–72. https://doi.org/10.3892/or.2011.1249.

75. Bolufer P, Barragan E, Collado M, Cervera J, López JA, Sanz MA. Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. Leuk Res. 2006;30(12):1471–91. https://doi.org/10.1016/j.leukres.2006.01.016.

76. Bruge F, Virgili S, Cacciamani T, Principi F, Tiano L, Littarru GP. NAD(P)H:quinone oxidoreductase (NQO1) loss of function in Burkitt's lymphoma cell lines. BioFactors (Oxford, England). 2008;32(1–4):71–81. https://doi.org/10.1002/biof.5520320109.

77. He H, Zhai X, Liu X, Zheng J, Zhai Y, Gao F, Chen Y, Lu J. Associations of NQO1 C609T and NQO1 C465T polymorphisms with acute leukemia risk: a PRISMA-compliant meta-analysis. Onco Targets Ther. 2017;10:1793–801. https://doi.org/10.2147/OTT.S132503.

78. Malik MA, Zargar SA, Mittal B. Role of NQO1 609C>T and NQO2 -3423G>A gene polymorphisms in esophageal cancer risk in Kashmir valley and meta analysis. Mol Biol Rep. 2012;39(9):9095–104. https://doi.org/10.1007/s11033-012-1781-y.

79. Saldivar SJ, Wang Y, Zhao H, Shao L, Lin J, Spitz MR, Wu X. An association between a NQO1 genetic polymorphism and risk of lung cancer. Mutat Res. 2005;582(1–2):71–8. https://doi.org/10.1016/j.mrgentox.2004.12.010.

80. Gaedigk A, Tyndale RF, Jurima-Romet M, Sellers EM, Grant DM, Leeder JS. NAD(P)H:quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations. Pharmacogenetics. 1998;8(4):305–13. https://doi.org/10.1097/00008571-199808000-00004.

81. Pey AL. Phenotypic modulation of cancer-associated antioxidant NQO1 activity by post-translational modifications and the natural diversity of the human genome. Antioxidants (Basel). 2023;12(2):379. https://doi.org/10.3390/antiox12020379.

82. Ziech D, Franco R, Pappa A, Panayiotidis MI. Reactive oxygen species (ROS)–induced genetic and epigenetic alterations in human carcinogenesis. Mutat Res. 2011;711(1–2):167–73. https://doi.org/10.1016/j.mrfmmm.2011.02.015.

83. Liu S, Pi J, Zhang Q. Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. Redox Biol. 2022;54: 102389. https://doi.org/10.1016/j.redox.2022.102389.

84. Vargas MR, Johnson JA. The Nrf2-ARE cytoprotective pathway in astrocytes. Expert Rev Mol Med. 2009;11: e17. https://doi.org/10.1017/S1462399409001094.

85. Gopinath K, Sudhandiran G. Naringin modulates oxidative stress and inflammation in 3-nitropropionic acid-induced neurodegeneration through the activation of nuclear factor-erythroid 2-related factor-2 signalling pathway. Neuroscience. 2012;227:134–43. https://doi.org/10.1016/j.neuroscience.2012.07.060.

86. Terai K, Dong GZ, Oh ET, Park MT, Gu Y, Song CW, Park HJ. Cisplatin enhances the anticancer effect of beta-lapachone by upregulating NQO1. Anticancer Drugs. 2009;20(10):901–9. https://doi.org/10.1097/CAD.0b013e328330098d.

87. Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. Oncogene. 1998;17(24):3145–56. https://doi.org/10.1038/sj.onc.1202237.

88. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. Free Radical Biol Med. 2015;88(Pt B):179–88. https://doi.org/10.1016/j.freeradbiomed.2015.04.036.

89. Wang W, Wang WP, Zhang GL, Wu YF, Xie T, Kan MC, Fang HB, Wang HC. Activation of Nrf2-ARE signal pathway in hippocampus of amygdala kindling rats. Neurosci Lett. 2013;543:58–63. https://doi.org/10.1016/j.neulet.2013.03.038.

90. Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA, Murphy TH. Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. J Neurosci. 2003;23(8):3394–406. https://doi.org/10.1523/JNEUROSCI.23-08-03394.2003.

91. Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1–Nrf2 pathway in stress response and cancer evolution. Genes Cells. 2011;16:123–40. https://doi.org/10.1111/j.1365-2443.2010.01473.x.

92. Kaspar JW, Jaiswal AK. Antioxidant-induced phosphorylation of tyrosine 486 leads to rapid nuclear export of Bach1 that allows Nrf2 to bind to the antioxidant response element and activate defensive gene expression. J Biol Chem. 2010;285(1):153–62. https://doi.org/10.1074/jbc.M109.040022.

93. Matzinger M, Fischhuber K, Pölöske D, Mechtler K, Heiss EH. AMPK leads to phosphorylation of the transcription factor Nrf2, tuning transactivation of selected target genes. Redox Biol. 2020;29: 101393. https://doi.org/10.1016/j.redox.2019.101393.

94. Mo C, Wang L, Zhang J, Numazawa S, Tang H, Tang X, Han X, Li J, Yang M, Wang Z, Wei D, Xiao H. The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice. Antioxid Redox Signal. 2014;20(4):574–88. https://doi.org/10.1089/ars.2012.5116.

95. Mo Y, Zhu JL, Jiang A, Zhao J, Ye L, Han B. Compound 13 activates AMPK-Nrf2 signaling to protect neuronal cells from oxygen glucose deprivation-reoxygenation. Aging. 2019;11(24):12032–42. https://doi.org/10.18632/aging.102534.

96. Weng B, Zhang X, Chu X, Gong X, Cai C. Nrf2-Keap1-ARE-NQO1 signaling attenuates hyperoxia-induced lung cell injury by inhibiting apoptosis. Mol Med Rep. 2021;23(3):221. https://doi.org/10.3892/mmr.2021.11860.

97. Ziady AG, Sokolow A, Shank S, Corey D, Myers R, Plafker S, Kelley TJ. Interaction with CREB binding protein modulates the activities of Nrf2 and NF-κB in cystic fibrosis airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2012;302(11):L1221–31. https://doi.org/10.1152/ajplung.00156.2011.

98. Yardım A, Kucukler S, Özdemir S, Çomaklı S, Caglayan C, Kandemir FM, Çelik H. Silymarin alleviates docetaxel-induced central and peripheral neurotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. Gene. 2021;769: 145239. https://doi.org/10.1016/j.gene.2020.145239.

99. Ge M, Yao W, Yuan D, Zhou S, Chen X, Zhang Y, Li H, Xia Z, Hei Z. Brg1-mediated Nrf2/HO-1 pathway activation alleviates hepatic ischemia-reperfusion injury. Cell Death Dis. 2017;8(6): e2841. https://doi.org/10.1038/cddis.2017.236.

100. Chen M, Lu Y, Zhou M, Wang W, Zheng M, Liu C. The protection impact of tectoridin on PC12 cell preventing OGD/R-caused damage through PI3K/AKT signaling channel. Eur J Pharmacol. 2023;941: 175491. https://doi.org/10.1016/j.ejphar.2023.175491.

101. Chen Y, Wu L, Bao M. MiR-485-5p Suppress the Malignant Characteristics of the Lung Adenocarcinoma via Targeting NADPH Quinone Oxidoreductase-1 to Inhibit the PI3K/Akt. Mol Biotechnol. 2023;65(5):794–806. https://doi.org/10.1007/s12033-022-00577-y.

102. Wang X, McCullough KD, Franke TF, Holbrook NJ. Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. J Biol Chem. 2000;275(19):14624–31. https://doi.org/10.1074/jbc.275.19.14624.

103. Ali T, Kim T, Rehman SU, Khan MS, Amin FU, Khan M, Ikram M, Kim MO. Natural dietary supplementation of anthocyanins via PI3K/Akt/Nrf2/HO-1 pathways mitigate oxidative stress, neurodegeneration, and memory impairment in a mouse model of Alzheimer's disease. Mol Neurobiol. 2018;55(7):6076–93. https://doi.org/10.1007/s12035-017-0798-6.

104. Zhuang Y, Wu H, Wang X, He J, He S, Yin Y. Resveratrol attenuates oxidative stress-induced intestinal barrier injury through PI3K/Akt-mediated Nrf2 signaling pathway. Oxid Med Cell Longev. 2019;2019:7591840. https://doi.org/10.1155/2019/7591840.

105. Gong G, Yin L, Yuan L, Sui D, Sun Y, Fu H, Chen L, Wang X. Ganglioside GM1 protects against high altitude cerebral edema in rats by suppressing the oxidative stress and inflammatory response via the PI3K/Akt-Nrf2 pathway. Mol Immunol. 2018;95:91–8.

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 23 of 28

106. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochem Biophys Acta. 2016;1863(12):2977–92. https://doi.org/10.1016/j.bbamcr.2016.09.012.

107. Papaiahgari S, Zhang Q, Kleeberger SR, Cho HY, Reddy SP. Hyperoxia stimulates an Nrf2-ARE transcriptional response via ROS-EGFR-PI3K-Akt/ERK MAP kinase signaling in pulmonary epithelial cells. Antioxid Redox Signal. 2006;8(1–2):43–52. https://doi.org/10.1089/ars.2006.8.43.

108. Li Y, Chen L, Zheng D, Liu JX, Liu C, Qi SH, Hu PC, Yang XF, Min JW. Echinocystic acid alleviated hypoxic-ischemic brain damage in neonatal mice by activating the PI3K/Akt/Nrf2 signaling pathway. Front Pharmacol. 2023;14:1103265. https://doi.org/10.3389/fphar.2023.1103265.

109. Saha S, Buttari B, Panieri E, Profumo E, Saso L. An overview of Nrf2 signaling pathway and its role in inflammation. Molecules (Basel, Switzerland). 2020;25(22):5474. https://doi.org/10.3390/molecules25225474.

110. Song MY, Lee DY, Chun KS, Kim EH. The role of NRF2/KEAP1 signaling pathway in cancer metabolism. Int J Mol Sci. 2021;22(9):4376. https://doi.org/10.3390/ijms22094376.

111. Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, Rumsey WL, Attucks OC, Franklin S, Levonen AL, Kensler TW, Dinkova-Kostova AT. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discovery. 2019;18(4):295–317. https://doi.org/10.1038/s41573-018-0008-x.

112. Adinolfi S, Patinen T, Jawahar Deen A, Pitkänen S, Härkönen J, Kansanen E, Küblbeck J, Levonen AL. The KEAP1-NRF2 pathway: targets for therapy and role in cancer. Redox Biol. 2023;63: 102726. https://doi.org/10.1016/j.redox.2023.102726.

113. Stringer JL, Gaikwad A, Gonzales BN, Long DJ Jr, Marks LM, Jaiswal AK. Presence and induction of the enzyme NAD(P)H: quinone oxidoreductase 1 in the central nervous system. J Comp Neurol. 2004;471(3):289–97. https://doi.org/10.1002/cne.20048.

114. Gussing F, Bohm S. NQO1 activity in the main and the accessory olfactory systems correlates with the zonal topography of projection maps. Eur J Neurosci. 2004;19(9):2511–8. https://doi.org/10.1111/j.0953-816X.2004.03331.x.

115. de Vries HE, Witte M, Hondius D, Rozemuller AJ, Drukarch B, Hoozemans J, van Horssen J. Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? Free Radical Biol Med. 2008;45(10):1375–83. https://doi.org/10.1016/j.freeradbiomed.2008.09.001.

116. Torres-Lista V, Parrado-Fernández C, Alvarez-Montón I, Frontiñán-Rubio J, Durán-Prado M, Peinado JR, Johansson B, Alcaín FJ, Giménez-Llort L. Neophobia, NQO1 and SIRT1 as premorbid and prodromal indicators of AD in 3xTg-AD mice. Behav Brain Res. 2014;271:140–6. https://doi.org/10.1016/j.bbr.2014.04.055.

117. Uruno A, Matsumaru D, Ryoke R, Saito R, Kadoguchi S, Saigusa D, Saito T, Saido TC, Kawashima R, Yamamoto M. Nrf2 suppresses oxidative stress and inflammation in app Knock-In Alzheimer's disease model mice. Mol Cell Biol. 2020;40(6):e00467-e519. https://doi.org/10.1128/MCB.00467-19.

118. Du Y, Liu G, Chen D, Yang J, Wang J, Sun Y, Zhang Q, Liu Y. NQO1 regulates expression and alternative splicing of apoptotic genes associated with Alzheimer's disease in PC12 cells. Brain Behav. 2023;13(5): e2917. https://doi.org/10.1002/brb3.2917.

119. Panja S, Siegel D, Camandola S, de Cabo R, Ross D, Mallela KMG. FAD-deficient P187S mutation of NAD(P)H:quinone oxidoreductase 1 (NQO1*2) binds and accelerates β-amyloid aggregation. 2022. Bioscience Rep. https://doi.org/10.1042/BSR20220643.

120. Martínez-Limón A, Alriquet M, Lang WH, Calloni G, Wittig I, Vabulas RM. Recognition of enzymes lacking bound cofactor by protein quality control. Proc Natl Acad Sci USA. 2016;113(43):12156–61. https://doi.org/10.1073/pnas.1611994113.

121. Sun Y, Huang J, Chen Y, Shang H, Zhang W, Yu J, He L, Xing C, Zhuang C. Direct inhibition of Keap1-Nrf2 protein-protein interaction as a potential therapeutic strategy for Alzheimer's disease. Bioorg Chem. 2020;103: 104172. https://doi.org/10.1016/j.bioorg.2020.104172.

122. Alcalde LA, de Freitas BS, Machado GDB, de Freitas Crivelaro PC, Dornelles VC, Gus H, Monteiro RT, Kist LW, Bogo MR, Schröder N. Iron chelator deferiprone rescues memory deficits, hippocampal BDNF levels and antioxidant defenses in an experimental model of memory impairment. Biometals. 2018;31(6):927–40. https://doi.org/10.1007/s10534-018-0135-1.

123. Cai Y, Xiao R, Zhang Y, Xu D, Wang N, Han M, Zhang Y, Zhang L, Zhou W. DHPA Protects SH-SY5Y cells from oxidative stress-induced apoptosis via mitochondria apoptosis and the keap1/Nrf2/HO-1 signaling pathway. Antioxidants (Basel, Switzerland). 2022;11(9):1794. https://doi.org/10.3390/antiox11091794.

124. SantaCruz KS, Yazlovitskaya E, Collins J, Johnson J, DeCarli C. Regional NAD(P)H:quinone oxidoreductase activity in Alzheimer's disease. Neurobiol Aging. 2004;25(1):63–9. https://doi.org/10.1016/s0197-4580(03)00117-9.

125. Raina AK, Templeton DJ, Deak JC, Perry G, Smith MA. Quinone reductase (NQO1), a sensitive redox indicator, is increased in Alzheimer's disease. Redox Rep. 1999;4(1–2):23–7. https://doi.org/10.1179/135100099101534701.

126. Wang Y, Santa-Cruz K, DeCarli C, Johnson JA. NAD(P)H:quinone oxidoreductase activity is increased in hippocampal pyramidal neurons of patients with Aalzheimer's disease. Neurobiol Aging. 2000;21(4):525–31. https://doi.org/10.1016/s0197-4580(00)00114-7.

127. Tsvetkov P, Adamovich Y, Elliott E, Shaul Y. E3 ligase STUB1/CHIP regulates NAD(P)H:quinone oxidoreductase 1 (NQO1) accumulation in aged brain, a process impaired in certain Alzheimer disease patients. J Biol Chem. 2011;286(11):8839–45. https://doi.org/10.1074/jbc.M110.193276.

128. Duara R, Barker W. Heterogeneity in Alzheimer's disease diagnosis and progression rates: implications for therapeutic trials. Neurotherapeutics. 2022;19(1):8–25. https://doi.org/10.1007/s13311-022-01185-z.

129. Wang B, Jin F, Xie Y, Tang Y, Kan R, Zheng C, Yang Z, Wang L. Association analysis of NAD(P)H:quinone oxidoreductase gene 609 C/T polymorphism with Alzheimer's disease. Neurosci Lett. 2006;409(3):179–81. https://doi.org/10.1016/j.neulet.2006.09.042.

130. Kaur K, Narang RK, Singh S. Role of Nrf2 in oxidative stress, neuroinflammation and autophagy in Alzheimer's disease: regulation of Nrf2 by different signaling pathways. Curr Mol Med. 2023. https://doi.org/10.2174/1566524023666230726145447.

131. Isidoro C, Biagioni F, Giorgi FS, Fulceri F, Paparelli A, Fornai F. The role of autophagy on the survival of dopamine neurons. Curr Top Med Chem. 2009;9(10):869–79. https://doi.org/10.2174/156802609789378281.

132. Shadfar S, Parakh S, Jamali MS, Atkin JD. Redox dysregulation as a driver for DNA damage and its relationship to neurodegenerative diseases. Translational Neurodegeneration. 2023;12(1):18. https://doi.org/10.1186/s40035-023-00350-4.

133. Son HJ, Choi JH, Lee JA, Kim DJ, Shin KJ, Hwang O. Induction of NQO1 and neuroprotection by a novel compound KMS04014 in Parkinson's disease models. J Mol Neurosci: MN. 2015;56(2):263–72. https://doi.org/10.1007/s12031-015-0516-7.

134. Hwang O. Role of oxidative stress in Parkinson's disease. Exp Neurobiol. 2013;22(1):11–7. https://doi.org/10.5607/en.2013.22.1.11.

135. Ishii T, Warabi E, Mann GE. Circadian control of p75 neurotrophin receptor leads to alternate activation of Nrf2 and c-Rel to reset energy metabolism in astrocytes via brain-derived neurotrophic factor. Free Radical Biol Med. 2018;119:34–44. https://doi.org/10.1016/j.freeradbiomed.2018.01.026.

136. Ishii T, Warabi E, Mann GE. Circadian control of BDNF-mediated Nrf2 activation in astrocytes protects dopaminergic neurons from ferroptosis. Free Radical Biol Med. 2019;133:169–78. https://doi.org/10.1016/j.freeradbiomed.2018.09.002.

137. Mencke P, Boussaad I, Romano CD, Kitami T, Linster CL, Krüger R. The Role of DJ-1 in Cellular Metabolism and Pathophysiological Implications for Parkinson's Disease. Cells. 2021;10(2):347. https://doi.org/10.3390/cells10020347.

138. Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc Natl Acad Sci USA. 2006;103(41):15091–6. https://doi.org/10.1073/pnas.0607260103.

139. van Muiswinkel FL, de Vos RA, Bol JG, Andringa G, Jansen Steur EN, Ross D, Siegel D, Drukarch B. Expression of NAD(P)H:quinone oxidoreductase in the normal and Parkinsonian substantia nigra. Neurobiol Aging. 2004;25(9):1253–62. https://doi.org/10.1016/j.neurobiolaging.2003.12.010.

140. Luo S, Kang SS, Wang ZH, Liu X, Day JX, Wu Z, Peng J, Xiang D, Springer W, Ye K. Akt Phosphorylates NQO1 and triggers its degradation,

Yuhan *et al. Journal of Translational Medicine*　　　(2024) 22:4

Page 24 of 28

abolishing its antioxidative activities in parkinson's disease. J Neurosci. 2019;39(37):7291–305. https://doi.org/10.1523/JNEUROSCI.0625-19.2019.

141. Lastres-Becker I, García-Yagüe AJ, Scannevin RH, Casarejos MJ, Kügler S, Rábano A, Cuadrado A. Repurposing the NRF2 activator dimethyl fumarate as therapy against synucleinopathy in Parkinson's Disease. Antioxid Redox Signal. 2016;25(2):61–77. https://doi.org/10.1089/ars.2015.6549.

142. Unni S, Deshmukh P, Krishnappa G, Bharath MMS, Padmanabhan B. Chlorhexidine as a Keap1-Nrf2 inhibitor: a new target for an old drug for Parkinson's disease therapy. J Biomol Struct Dyn. 2022. https://doi.org/10.1080/07391102.2022.2086175.

143. Dai D, Lin P, Wang Y, Zhou X, Tao J, Jiang D, Zhou H, Ru P, Pan G, Li J, Zhang Y, Yin H, Duan S. Association of NQO1 and TNF polymorphisms with Parkinson's disease: a meta-analysis of 15 genetic association studies. Biomed Rep. 2014;2(5):713–8. https://doi.org/10.3892/br.2014.296.

144. Langston JW, Ballard PA Jr. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. N Engl J Med. 1983;309(5):310. https://doi.org/10.1056/nejm198308043090511.

145. Mizuno Y, Sone N, Suzuki K, Saitoh T. Studies on the toxicity of 1-methyl-4-phenylpyridinium ion (MPP+) against mitochondria of mouse brain. J Neurol Sci. 1988;86(1):97–110. https://doi.org/10.1016/0022-510x(88)90010-x.

146. Speciale SG. MPTP: insights into parkinsonian neurodegeneration. Neurotoxicol Teratol. 2002;24(5):607–20. https://doi.org/10.1016/s0892-0362(02)00222-2.

147. Gille G, Hung ST, Reichmann H, Rausch WD. Oxidative stress to dopaminergic neurons as models of Parkinson's disease. Ann N Y Acad Sci. 2004;1018:533–40. https://doi.org/10.1196/annals.1296.066.

148. Langston JW, Ballard P. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. Can J Neurol Sci. 1984;11(1):160–5. https://doi.org/10.1017/s0317167100046333.

149. Bai X, Bian Z, Zhang M. Targeting the Nrf2 signaling pathway using phytochemical ingredients: A novel therapeutic road map to combat neurodegenerative diseases. Phytomedicine. 2023;109:154582. https://doi.org/10.1016/j.phymed.2022.154582.

150. Han JM, Lee YJ, Lee SY, Kim EM, Moon Y, Kim HW, Hwang O. Protective effect of sulforaphane against dopaminergic cell death. J Pharmacol Exp Ther. 2007;321(1):249–56. https://doi.org/10.1124/jpet.106.110866.

151. Lee JA, Son HJ, Kim JH, Park KD, Shin N, Kim HR, Kim EM, Kim DJ, Hwang O. A novel synthetic isothiocyanate ITC-57 displays antioxidant, anti-inflammatory, and neuroprotective properties in a mouse Parkinson's disease model. Free Radical Res. 2016;50(11):1188–99. https://doi.org/10.1080/10715762.2016.1223293.

152. Kamal RM, Abdull Razis AF, Mohd Sukri NS, Perimal EK, Ahmad H, Patrick R, Djedaini-Pilard F, Mazzon E, Rigaud S. Beneficial health effects of glucosinolates-derived isothiocyanates on cardiovascular and neurodegenerative diseases. Molecules (Basel, Switzerland). 2022;27(3):624. https://doi.org/10.3390/molecules27030624.

153. Chiu YJ, Lin CH, Lin CY, Yang PN, Lo YS, Chen YC, Chen CM, Wu YR, Yao CF, Chang KH, Lee-Chen GJ. Investigating therapeutic effects of indole derivatives targeting inflammation and oxidative stress in neurotoxin-induced cell and mouse models of Parkinson's disease. Int J Mol Sci. 2023;24(3):2642. https://doi.org/10.3390/ijms24032642.

154. Huang L, Han Y, Zhou Q, Sun Z, Yan J. Isoliquiritigenin attenuates neuroinflammation in mice model of Parkinson's disease by promoting Nrf2/NQO-1 pathway. Transl Neurosci. 2022;13(1):301–8. https://doi.org/10.1515/tnsci-2022-0239.

155. Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, Cheng CW. Pesticide exposure on southwestern Taiwanese with MnSOD and NQO1 polymorphisms is associated with increased risk of Parkinson's disease. Clinica chimica acta. 2007;378(1–2):136–41. https://doi.org/10.1016/j.cca.2006.11.006.

156. Ahmed H, Abushouk AI, Gabr M, Negida A, Abdel-Daim MM. Parkinson's disease and pesticides: a meta-analysis of disease connection and genetic alterations. Biomed Pharmacother. 2017;90:638–49. https://doi.org/10.1016/j.biopha.2017.03.100.

157. Stavropoulou C, Zachaki S, Alexoudi A, Chatzi I, Georgakakos VN, Terzoudi GI, Pantelias GE, Karageorgiou CE, Sambani C. The C609T inborn polymorphism in NAD(P)H:quinone oxidoreductase 1 is associated with susceptibility to multiple sclerosis and affects the risk of development

158. Shimizu F, Ogawa R, Mizukami Y, Watanabe K, Hara K, Kadono C, Takahashi T, Misu T, Takeshita Y, Sano Y, Fujisawa M, Maeda T, Nakashima I, Fujihara K, Kanda T. GRP78 antibodies are associated with blood-brain barrier breakdown in anti-myelin oligodendrocyte glycoprotein antibody-associated disorder. Neurol Neuroimmunol Neuroinflamm. 2021;9(1):e1038. https://doi.org/10.1212/NXI.0000000000001038.

159. Voigt D, Scheidt U, Derfuss T, Brück W, Junker A. Expression of the antioxidative enzyme peroxiredoxin 2 in multiple sclerosis lesions in relation to inflammation. Int J Mol Sci. 2017;18(4):760. https://doi.org/10.3390/ijms18040760.

160. Agúndez JA, García-Martín E, Martínez C, Benito-León J, Millán-Pascual J, Calleja P, Díaz-Sánchez M, Pisa D, Turpín-Fenoll L, Alonso-Navarro H, Ayuso-Peralta L, Torrecillas D, Plaza-Nieto JF, Jiménez-Jiménez FJ. NQO1 gene rs1800566 variant is not associated with risk for multiple sclerosis. BMC Neurol. 2014;14:87. https://doi.org/10.1186/1471-2377-14-87.

161. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, Tanaka N, Moriguchi T, Motohashi H, Nakayama K, Yamamoto M. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624. https://doi.org/10.1038/ncomms11624.

162. Higashi C, Kawaji A, Tsuda N, Hayashi M, Saito R, Yagishita Y, Suzuki T, Uruno A, Nakamura M, Nakao K, Furusako S, Yamamoto M. The novel Nrf2 inducer TFM-735 ameliorates experimental autoimmune encephalomyelitis in mice. Eur J Pharmacol. 2017;802:76–84. https://doi.org/10.1016/j.ejphar.2017.02.044.

163. Garcia-Mesa Y, Xu HN, Vance P, Gruenewald AL, Garza R, Midkiff C, Alvarez-Hernandez X, Irwin DJ, Gill AJ, Kolson DL. Dimethyl fumarate, an approved multiple sclerosis treatment, reduces brain oxidative stress in siv-infected rhesus macaques: potential therapeutic repurposing for HIV neuroprotection. Antioxidants (Basel, Switzerland). 2021;10(3):416. https://doi.org/10.3390/antiox10030416.

164. Scuderi SA, Ardizzone A, Paterniti I, Esposito E, Campolo M. Antioxidant and anti-inflammatory effect of Nrf2 inducer dimethyl fumarate in neurodegenerative diseases. Antioxidants (Basel, Switzerland). 2020;9(7):630. https://doi.org/10.3390/antiox9070630.

165. Linker RA, Lee DH, Ryan S, van Dam AM, Conrad R, Bista P, Zeng W, Hronowsky X, Buko A, Chollate S, Ellrichmann G, Brück W, Dawson K, Goelz S, Wiese S, Scannevin RH, Lukashev M, Gold R. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain. 2011;134(Pt 3):678–92. https://doi.org/10.1093/brain/awq386.

166. Li L, Dong H, Song E, Xu X, Liu L, Song Y. Nrf2/ARE pathway activation, HO-1 and NQO1 induction by polychlorinated biphenyl quinone is associated with reactive oxygen species and PI3K/AKT signaling. Chem Biol Interact. 2014;209:56–67. https://doi.org/10.1016/j.cbi.2013.12.005.

167. Long Ting, et al. "Neuroprotective effects of melatonin on experimental allergic encephalomyelitis mice via anti-oxidative stress activity. J Mol Neurosci. 2018;64(2):233–41. https://doi.org/10.1007/s12031-017-1022-x.

168. Zhang Q, Li Z, Wu S, Li X, Sang Y, Li J, Niu Y, Ding H. Myricetin alleviates cuprizone-induced behavioral dysfunction and demyelination in mice by Nrf2 pathway. Food Funct. 2016;7(10):4332–42. https://doi.org/10.1039/c6fo00825a.

169. Hammer A, Waschbisch A, Kuhbandner K, Bayas A, Lee DH, Duscha A, Haghikia A, Gold R, Linker RA. The NRF2 pathway as potential biomarker for dimethyl fumarate treatment in multiple sclerosis. Ann Clin Transl Neurol. 2018;5(6):668–76. https://doi.org/10.1002/acn3.553.

170. Gopal S, Mikulskis A, Gold R, Fox RJ, Dawson KT, Amaravadi L. Evidence of activation of the Nrf2 pathway in multiple sclerosis patients treated with delayed-release dimethyl fumarate in the Phase 3 DEFINE and CONFIRM studies. Multiple Scler (Houndmills, Basingstoke, England). 2017;23(14):1875–83. https://doi.org/10.1177/1352458517690617.

171. Alexoudi A, Zachaki S, Stavropoulou C, Gavrili S, Spiliopoulou C, Papadodima S, Karageorgiou CE, Sambani C. Possible implication of GSTP1 and NQO1 polymorphisms on natalizumab response in multiple sclerosis. Ann Clin Lab Sci. 2016;46(6):586–91.

172. Lim JL, van der Pol SM, Di Dio F, van Het Hof B, Kooij G, de Vries HE, van Horssen J. Protective effects of monomethyl fumarate at the inflamed

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 25 of 28

blood-brain barrier. Microvasc Res. 2016;105:61–9. https://doi.org/10.1016/j.mvr.2015.12.003.

173. Zarzuelo Romero MJ, Pérez Ramírez C, Carrasco Campos MI, Sánchez Martín A, Calleja Hernández MÁ, Ramírez Tortosa MC, Jiménez Morales A. Therapeutic value of single nucleotide polymorphisms on the efficacy of new therapies in patients with multiple sclerosis. J Personalized Med. 2021;11(5):335. https://doi.org/10.3390/jpm11050335.

174. Leonard MO, Kieran NE, Howell K, Burne MJ, Varadarajan R, Dhakshinamoorthy S, Porter AG, O'Farrelly C, Rabb H, Taylor CT. Reoxygenation-specific activation of the antioxidant transcription factor Nrf2 mediates cytoprotective gene expression in ischemia-reperfusion injury. FASEB J. 2006;20(14):2624–6. https://doi.org/10.1096/fj.06-5097fje.

175. Alfieri A, Srivastava S, Siow RC, Modo M, Fraser PA, Mann GE. Targeting the Nrf2-Keap1 antioxidant defence pathway for neurovascular protection in stroke. J Physiol. 2011;589(17):4125–36. https://doi.org/10.1113/jphysiol.2011.210294.

176. Yan L, Xu D, Xiao Y, Dai M, Wang T, Zhuang X, Wu K. Genetic polymorphism of NQO1 gene is associated with susceptibility of ischemic stroke in Chinese han nationality. Neuropsychiatr Dis Treat. 2022;18:2211–7. https://doi.org/10.2147/NDT.S379742.

177. Zhang X, Wu Q, Wang Z, Li H, Dai J. Keap1-Nrf2/ARE signal pathway activated by butylphthalide in the treatment of ischemic stroke. Am J Transl Res. 2022;14(4):2637–46.

178. Hou X, Xu H, Chen W, Zhang N, Zhao Z, Fang X, Zhang X, Chen H, Xu Y. Neuroprotective effect of dimethyl fumarate on cognitive impairment induced by ischemic stroke. Ann Transl Med. 2020;8(6):375. https://doi.org/10.21037/atm.2020.02.10.

179. Christopher E, Loan JJM, Samarasekera N, McDade K, Rose J, Barrington J, Hughes J, Smith C, Al-Shahi Salman R. Nrf2 activation in the human brain after stroke due to supratentorial intracerebral haemorrhage: a case-control study. BMJ Neurol open. 2022;4(1): e000238. https://doi.org/10.1136/bmjno-2021-000238.

180. Lin R, Cai J, Kostuk EW, Rosenwasser R, Iacovitti L. Fumarate modulates the immune/inflammatory response and rescues nerve cells and neurological function after stroke in rats. J Neuroinflamm. 2016;13(1):269. https://doi.org/10.1186/s12974-016-0733-1.

181. Li Y, Chu L, Liu C, Zha Z, Shu Y. Protective effect of GSK-3β/Nrf2 mediated by dimethyl fumarate in middle cerebral artery embolization reperfusion rat model. Curr Neurovasc Res. 2021;18(4):456–64. https://doi.org/10.2174/1567202618666211109105024.

182. Griffin P, Dimitry JM, Sheehan PW, Lananna BV, Guo C, Robinette ML, Hayes ME, Cedeño MR, Nadarajah CJ, Ezerskiy LA, Colonna M, Zhang J, Bauer AQ, Burris TP, Musiek ES. Circadian clock protein Rev-erbα regulates neuroinflammation. Proc Natl Acad Sci USA. 2019;116(11):5102–7. https://doi.org/10.1073/pnas.1812405116.

183. Sheng M, Chen X, Yu Y, Wu Q, Kou J, Chen G. Rev-erbα agonist SR9009 protects against cerebral ischemic injury through mechanisms involving Nrf2 pathway. Front Pharmacol. 2023;14:1102567. https://doi.org/10.3389/fphar.2023.1102567.

184. Chen N, et al. The role of Cullin 3 in cerebral ischemia-reperfusion injury. Neuroscience. 2023;514:14–24. https://doi.org/10.1016/j.neuroscience.2023.01.027.

185. Mann GE. Nrf2-mediated redox signalling in vascular health and disease. Free Radical Biol Med. 2014;75(Suppl 1):S1. https://doi.org/10.1016/j.freeradbiomed.2014.10.595.

186. Warpsinski G, Smith MJ, Srivastava S, Keeley TP, Siow RCM, Fraser PA, Mann GE. Nrf2-regulated redox signaling in brain endothelial cells adapted to physiological oxygen levels: consequences for sulforaphane mediated protection against hypoxia-reoxygenation. Redox Biol. 2020;37: 101708. https://doi.org/10.1016/j.redox.2020.101708.

187. Soane L, Li Dai W, Fiskum G, Bambrick LL. Sulforaphane protects immature hippocampal neurons against death caused by exposure to hemin or to oxygen and glucose deprivation. J Neurosci Res. 2010;88(6):1355–63. https://doi.org/10.1002/jnr.22307.

188. Wu J, Li R, Li W, Ren M, Thangthaeng N, Sumien N, Liu R, Yang S, Simpkins JW, Forster MJ, Yan LJ. Administration of 5-methoxyindole-2-carboxylic acid that potentially targets mitochondrial dihydrolipoamide dehydrogenase confers cerebral preconditioning against ischemic stroke injury. Free Radical Biol Med. 2017;113:244–54. https://doi.org/10.1016/j.freeradbiomed.2017.10.008.

189. Neves LT, Paz LV, Wieck A, Mestriner RG, de Miranda Monteiro VAC, Xavier LL. Environmental enrichment in stroke research: an update. Transl Stroke Res. 2023. https://doi.org/10.1007/s12975-023-01132-w.

190. Zhang X, Yuan M, Yang S, Chen X, Wu J, Wen M, Yan K, Bi X. Enriched environment improves post-stroke cognitive impairment and inhibits neuroinflammation and oxidative stress by activating Nrf2-ARE pathway. Int J Neurosci. 2021;131(7):641–9. https://doi.org/10.1080/00207454.2020.1797722.

191. Zhang F, Lei F, Xiao X. Knockdown of CBX7 inhibits ferroptosis in rats with cerebral ischemia and improves cognitive dysfunction by activating the Nrf2/HO-1 pathway. J Biosci. 2022;47:40.

192. Chen G, Fang Q, Zhang J, Zhou D, Wang Z. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. J Neurosci Res. 2011;89(4):515–23. https://doi.org/10.1002/jnr.22577.

193. Zhang J, Zhu Y, Zhou D, Wang Z, Chen G. Recombinant human erythropoietin (rhEPO) alleviates early brain injury following subarachnoid hemorrhage in rats: possible involvement of Nrf2-ARE pathway. Cytokine. 2010;52(3):252–7. https://doi.org/10.1016/j.cyto.2010.08.011.

194. Wu Q, Zhang XS, Wang HD, Zhang X, Yu Q, Li W, Zhou ML, Wang XL. Astaxanthin activates nuclear factor erythroid-related factor 2 and the antioxidant responsive element (Nrf2-ARE) pathway in the brain after subarachnoid hemorrhage in rats and attenuates early brain injury. Mar Drugs. 2014;12(12):6125–41. https://doi.org/10.3390/md12126125.

195. Zhang Y, Sun J, Zhu S, Xu T, Lu J, Han H, Zhou C, Yan J. The role of rhynchophylline in alleviating early brain injury following subarachnoid hemorrhage in rats. Brain Res. 2016;1631:92–100. https://doi.org/10.1016/j.brainres.2015.11.035.

196. Xiao X, Sun S, Li Y, Cen X, Wu S, Lu A, Cai J, Zhao J, Li S. Geniposide attenuates early brain injury by inhibiting oxidative stress and neurocyte apoptosis after subarachnoid hemorrhage in rats. Mol Biol Rep. 2022;49(7):6303–11. https://doi.org/10.1007/s11033-022-07438-6.

197. Tsai TH, Lin SH, Wu CH, Tsai YC, Yang SF, Lin CL. Mechanisms and therapeutic implications of RTA 408, an activator of Nrf2, in subarachnoid hemorrhage-induced delayed cerebral vasospasm and secondary brain injury. PLoS ONE. 2020;15(10): e0240122. https://doi.org/10.1371/journal.pone.0240122.

198. Wang Z, Ji C, Wu L, Qiu J, Li Q, Shao Z, Chen G. Tert-butylhydroquinone alleviates early brain injury and cognitive dysfunction after experimental subarachnoid hemorrhage: role of Keap1/Nrf2/ARE pathway. PLoS ONE. 2014;9(5): e97685. https://doi.org/10.1371/journal.pone.0097685.

199. Hausburg MA, Banton KL, Roman PE, Salgado F, Baek P, Waxman MJ, Tanner A 2nd, Yoder J, Bar-Or D. Effects of propofol on ischemia-reperfusion and traumatic brain injury. J Crit Care. 2020;56:281–7. https://doi.org/10.1016/j.jcrc.2019.12.021.

200. Zhang HB, Tu XK, Chen Q, Shi SS. Propofol reduces inflammatory brain injury after subarachnoid hemorrhage: involvement of PI3K/Akt Pathway. J Stroke Cerebrovasc Dis. 2019;28(12): 104375. https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.104375.

201. Zolnourian A, Galea I, Bulters D. Neuroprotective role of the Nrf2 pathway in subarachnoid haemorrhage and its therapeutic potential. Oxid Med Cell Longev. 2019;2019:6218239. https://doi.org/10.1155/2019/6218239.

202. Ryan AK, Rich W, Reilly MA. Oxidative stress in the brain and retina after traumatic injury. Front Neurosci. 2023;17:1021152. https://doi.org/10.3389/fnins.2023.1021152.

203. Wu AG, Yong YY, Pan YR, Zhang L, Wu JM, Zhang Y, Tang Y, Wei J, Yu L, Law BY, Yu CL, Liu J, Lan C, Xu RX, Zhou XG, Qin DL. Targeting Nrf2-mediated oxidative stress response in traumatic brain injury: therapeutic perspectives of phytochemicals. Oxid Med Cell Longev. 2022;2022:1015791. https://doi.org/10.1155/2022/1015791.

204. Petersen A, Soderstrom M, Saha B, Sharma P. Animal models of traumatic brain injury: a review of pathophysiology to biomarkers and treatments. Exp Brain Res. 2021;239(10):2939–50. https://doi.org/10.1007/s00221-021-06178-6.

205. Bhowmick S, D'Mello V, Caruso D, Abdul-Muneer PM. Traumatic brain injury-induced downregulation of Nrf2 activates inflammatory response and apoptotic cell death. J Mol Med (Berl). 2019;97(12):1627–41. https://doi.org/10.1007/s00109-019-01851-4.

206. Chandran R, Kim T, Mehta SL, Udho E, Chanana V, Cengiz P, Kim H, Kim C, Vemuganti R. A combination antioxidant therapy to inhibit NOX2 and activate Nrf2 decreases secondary brain damage and improves

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 26 of 28

functional recovery after traumatic brain injury. J Cereb Blood Flow Metab. 2018;38(10):1818–27. https://doi.org/10.1177/0271678X17738701.

207. MacManus DB, Ghajari M. Material properties of human brain tissue suitable for modelling traumatic brain injury. Brain Multiphys. 2022;3:100059. https://doi.org/10.1016/j.brain.2022.100059.

208. He Y, Yan H, Ni H, Liang W, Jin W. Expression of nuclear factor erythroid 2-related factor 2 following traumatic brain injury in the human brain. NeuroReport. 2019;30(5):344–9. https://doi.org/10.1097/WNR.0000000000001205.

209. Zhang L, Wang H. Targeting the NF-E2-related factor 2 pathway: a novel strategy for traumatic brain injury. Mol Neurobiol. 2018;55(2):1773–85. https://doi.org/10.1007/s12035-017-0456-z.

210. Zhao J, Moore AN, Redell JB, Dash PK. Enhancing expression of Nrf2-driven genes protects the blood brain barrier after brain injury. J Neurosci. 2007;27(38):10240–8. https://doi.org/10.1523/JNEUROSCI.1683-07.2007.

211. Dong W, Yang B, Wang L, Li B, Guo X, Zhang M, Jiang Z, Fu J, Pi J, Guan D, Zhao R. Curcumin plays neuroprotective roles against traumatic brain injury partly via Nrf2 signaling. Toxicol Appl Pharmacol. 2018;346:28–36. https://doi.org/10.1016/j.taap.2018.03.020.

212. Li F, Wang X, Zhang Z, Zhang X, Gao P. Dexmedetomidine attenuates neuroinflammatory-induced apoptosis after traumatic brain injury via Nrf2 signaling pathway. Ann Clin Transl Neurol. 2019;6(9):1825–35. https://doi.org/10.1002/acn3.50878.

213. Huang GR, Hao FG. Dexmedetomidine Inhibits inflammation to alleviate early neuronal injury via TLR4/NF-κB pathway in rats with traumatic brain injury. Crit Rev Eukaryot Gene Expr. 2021;31(1):41–7. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2021037390.

214. Zhang ZW, Liang J, Yan JX, Ye YC, Wang JJ, Chen C, Sun HT, Chen F, Tu Y, Li XH. TBHQ improved neurological recovery after traumatic brain injury by inhibiting the overactivation of astrocytes. Brain Res. 2020;1739:146818. https://doi.org/10.1016/j.brainres.2020.146818.

215. Zhou J, Wang H, Shen R, Fang J, Yang Y, Dai W, Zhu Y, Zhou M. Mitochondrial-targeted antioxidant MitoQ provides neuroprotection and reduces neuronal apoptosis in experimental traumatic brain injury possibly via the Nrf2-ARE pathway. Am J Transl Res. 2018;10(6):1887–99.

216. Jin W, Kong J, Lu T, Wang H, Ni H, Wu J, Dai Y, Jiang J, Liang W. Erythropoietin prevents secondary brain injury induced by cortical lesion in mice: possible involvement of Nrf2 signaling pathway. Ann Clin Lab Sci. 2011;41(1):25–32.

217. Guo P, Jin Z, Wang J, Sang A, Wu H. Irisin rescues blood-brain barrier permeability following traumatic brain injury and contributes to the neuroprotection of exercise in traumatic brain injury. Oxid Med Cell Longev. 2021;2021:1118981. https://doi.org/10.1155/2021/1118981.

218. Yan X, Liu X, Wang Y, Ren X, Ma J, Song R, Wang X, Dong Y, Fan Q, Wei J, Yu A, Sui H, She G. Multi-omics integration reveals the hepatoprotective mechanisms of ursolic acid intake against chronic alcohol consumption. Eur J Nutr. 2022;61(1):115–26. https://doi.org/10.1007/s00394-021-02632-x.

219. Volmering E, Niehusmann P, Peeva V, Grote A, Zsurka G, Altmüller J, Nürnberg P, Becker AJ, Schoch S, Elger CE, Kunz WS. Neuropathological signs of inflammation correlate with mitochondrial DNA deletions in mesial temporal lobe epilepsy. Acta Neuropathol. 2016;132(2):277–88. https://doi.org/10.1007/s00401-016-1561-1.

220. Aguiar CC, Almeida AB, Araújo PV, de Abreu RN, Chaves EM, do Vale OC, Macêdo DS, Woods DJ, Fonteles MM, Vasconcelos SM. Oxidative stress and epilepsy: literature review. Oxid Med Cell Longev. 2012. https://doi.org/10.1155/2012/795259.

221. Mazzuferi M, Kumar G, van Eyll J, Danis B, Foerch P, Kaminski RM. Nrf2 defense pathway: experimental evidence for its protective role in epilepsy. Ann Neurol. 2013;74(4):560–8. https://doi.org/10.1002/ana.23940.

222. Kishore M, Pradeep M, Narne P, Jayalakshmi S, Panigrahi M, Patil A, Babu PP. Regulation of Keap1-Nrf2 axis in temporal lobe epilepsy-hippocampal sclerosis patients may limit the seizure outcomes. Neurol Sci. 2023. https://doi.org/10.1007/s10072-023-06936-0.

223. Kovac S, Dinkova Kostova AT, Herrmann AM, Melzer N, Meuth SG, Gorji A. Metabolic and homeostatic changes in seizures and acquired epilepsy-mitochondria, calcium dynamics and reactive oxygen species. Int J Mol Sci. 2017;18(9):1935. https://doi.org/10.3390/ijms18091935.

224. Radjendirane V, Joseph P, Lee YH, Kimura S, Klein-Szanto AJ, Gonzalez FJ, Jaiswal AK. Disruption of the DT diaphorase (NQO1) gene in mice leads to increased menadione toxicity. J Biol Chem. 1998;273(13):7382–9. https://doi.org/10.1074/jbc.273.13.7382.

225. Lapehn S, Piorczynski TB, Hansen JM, Harris C. Spatiotemporal evaluation of the mouse embryonic redox environment and histiotrophic nutrition following treatment with valproic acid and 1,2-dithiole-3-thione during early organogenesis. Reprod Toxicol. 2021;101:81–92. https://doi.org/10.1016/j.reprotox.2021.03.001.

226. Cao H, Zhang L, Qu Z, Tian S, Wang Z, Jiang Y, Hou Q, Jia L, Wang W. The protective effect of hydroxylated fullerene pretreatment on pilocarpine-induced status epilepticus. Brain Res. 2021;1764: 147468. https://doi.org/10.1016/j.brainres.2021.147468.

227. Singh N, Saha L, Kumari P, Singh J, Bhatia A, Banerjee D, Chakrabarti A. Effect of dimethyl fumarate on neuroinflammation and apoptosis in pentylenetetrazol kindling model in rats. Brain Res Bull. 2019;144:233–45. https://doi.org/10.1016/j.brainresbull.2018.11.013.

228. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. Nat Rev Neurol. 2019;15(8):459–72. https://doi.org/10.1038/s41582-019-0217-x.

229. Hu QP, Yan HX, Peng F, Feng W, Chen FF, Huang XY, Zhang X, Zhou YY, Chen YS. Genistein protects epilepsy-induced brain injury through regulating the JAK2/STAT3 and Keap1/Nrf2 signaling pathways in the developing rats. Eur J Pharmacol. 2021;912: 174620. https://doi.org/10.1016/j.ejphar.2021.174620.

230. Prakash C, Mishra M, Kumar P, Kumar V, Sharma D. Dehydroepiandrosterone alleviates oxidative stress and apoptosis in iron-induced epilepsy via activation of Nrf2/ARE signal pathway. Brain Res Bull. 2019;153:181–90. https://doi.org/10.1016/j.brainresbull.2019.08.019.

231. Willmore LJ, Sypert GW, Munson JB. Recurrent seizures induced by cortical iron injection: a model of posttraumatic epilepsy. Ann Neurol. 1978;4(4):329–36. https://doi.org/10.1002/ana.410040408.

232. Wang Z, Bai Z, Qin X, Cheng Y. Aberrations in oxidative stress markers in amyotrophic lateral sclerosis: a systematic review and meta-analysis. Oxid Med Cell Longev. 2019. https://doi.org/10.1155/2019/1712323.

233. Liu D, Wen J, Liu J, Li L. The roles of free radicals in amyotrophic lateral sclerosis: reactive oxygen species and elevated oxidation of protein, DNA, and membrane phospholipids. FASEB J. 1999;13(15):2318–28. https://doi.org/10.1096/fasebj.13.15.2318.

234. Petri S, Körner S, Kiaei M. Nrf2/ARE signaling pathway: key mediator in oxidative stress and potential therapeutic target in ALS. Neurol Res Int. 2012;2012: 878030. https://doi.org/10.1155/2012/878030.

235. Sarlette A, Krampfl K, Grothe C, Neuhoff NV, Dengler R, Petri S. Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 2008;67(11):1055–62. https://doi.org/10.1097/NEN.0b013e31818b4906.

236. Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M, Liby KT, Risingsong R, Sporn M, Beal MF, Kiaei M. Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free Radical Biol Med. 2011;51(1):88–96. https://doi.org/10.1016/j.freeradbiomed.2011.03.027.

237. Provenzano F, Nyberg S, Giunti D, Torazza C, Parodi B, Bonifacino T, Usai C, Kerlero de Rosbo N, Milanese M, Uccelli A, Shaw PJ, Ferraiuolo L, Bonanno G. Micro-RNAs shuttled by extracellular vesicles secreted from mesenchymal stem cells dampen astrocyte pathological activation and support neuroprotection in in-vitro models of ALS. Cells. 2022;11(23):3923. https://doi.org/10.3390/cells11233923.

238. Guo Y, Zhang Y, Wen D, Duan W, An T, Shi P, Wang J, Li Z, Chen X, Li C. The modest impact of transcription factor Nrf2 on the course of disease in an ALS animal model. Lab Invest. 2013;93(7):825–33. https://doi.org/10.1038/labinvest.2013.73.

239. Lastres-Becker I, Porras G, Arribas-Blázquez M, Maestro I, Borrego-Hernández D, Boya P, Cerdán S, García-Redondo A, Martínez A, Martin-Requero Á. Molecular alterations in sporadic and SOD1-ALS immortalized lymphocytes: towards a personalized therapy. Int J Mol Sci. 2021;22(6):3007. https://doi.org/10.3390/ijms22063007.

240. Martín-Cámara O, Arribas M, Wells G, Morales-Tenorio M, Martín-Requero Á, Porras G, Martínez A, Giorgi G, López-Alvarado P, Lastres-Becker I, Menéndez JC. Multitarget hybrid fasudil derivatives as a new

Yuhan *et al. Journal of Translational Medicine*   (2024) 22:4

Page 27 of 28

approach to the potential treatment of amyotrophic lateral sclerosis. J Med Chem. 2022;65(3):1867–82. https://doi.org/10.1021/acs.jmedchem.1c01255.

241. Volonté C, Amadio S. Rethinking puringergic concepts and updating the emerging role of P2X7 and P2X4 in amyotrophic lateral sclerosis. Neuropharmacology. 2022;221: 109278. https://doi.org/10.1016/j.neuropharm.2022.109278.

242. Ly D, Dongol A, Cuthbertson P, Guy TV, Geraghty NJ, Sophocleous RA, Sin L, Turner BJ, Watson D, Yerbury JJ, Sluyter R. The P2X7 receptor antagonist JNJ-47965567 administered thrice weekly from disease onset does not alter progression of amyotrophic lateral sclerosis in SOD1G93A mice. Purinergic Signalling. 2020;16(1):109–22. https://doi.org/10.1007/s11302-020-09692-4.

243. Deng Z, Lim J, Wang Q, Purtell K, Wu S, Palomo GM, Tan H, Manfredi G, Zhao Y, Peng J, Hu B, Chen S, Yue Z. ALS-FTLD-linked mutations of SQSTM1/p62 disrupt selective autophagy and NFE2L2/NRF2 antioxidative stress pathway. Autophagy. 2020;16(5):917–31. https://doi.org/10.1080/15548627.2019.1644076.

244. Mackenzie IR, Rademakers R. The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. Curr Opin Neurol. 2008;21(6):693–700. https://doi.org/10.1097/WCO.0b013e3283168d1d.

245. Tian YP, Che FY, Su QP, Lu YC, You CP, Huang LM, Wang SG, Wang L, Yu JX. Effects of mutant TDP-43 on the Nrf2/ARE pathway and protein expression of MafK and JDP2 in NSC-34 cells. Genet Mol Res. 2017. https://doi.org/10.4238/gmr16029638.

246. Liu C, Leng B, Li Y, Jiang H, Duan W, Guo Y, Li C, Hong K. Diallyl trisulfide protects motor neurons from the neurotoxic protein TDP-43 via activating lysosomal degradation and the antioxidant response. Neurochem Res. 2018;43(12):2304–12. https://doi.org/10.1007/s11064-018-2651-3.

247. Duan W, Li X, Shi J, Guo Y, Li Z, Li C. Mutant TAR DNA-binding protein-43 induces oxidative injury in motor neuron-like cell. Neuroscience. 2010;169(4):1621–9. https://doi.org/10.1016/j.neuroscience.2010.06.018.

248. Qi X, Jha SK, Jha NK, Dewanjee S, Dey A, Deka R, Pritam P, Ramgopal K, Liu W, Hou K. Antioxidants in brain tumors: current therapeutic significance and future prospects. Mol Cancer. 2022;21(1):204. https://doi.org/10.1186/s12943-022-01668-9.

249. Seng S, Avraham HK, Birrane G, Jiang S, Avraham S. Nuclear matrix protein (NRP/B) modulates the nuclear factor (Erythroid-derived 2)-related 2 (NRF2)-dependent oxidative stress response. J Biol Chem. 2010;285(34):26190–8. https://doi.org/10.1074/jbc.M109.095786.

250. Scuto M, Ontario ML, Salinaro AT, Caligiuri I, Rampulla F, Zimbone V, Modafferi S, Rizzolio F, Canzonieri V, Calabrese EJ, Calabrese V. Redox modulation by plant polyphenols targeting vitagenes for chemoprevention and therapy: relevance to novel anti-cancer interventions and mini-brain organoid technology. Free Radical Biol Med. 2022;179:59–75. https://doi.org/10.1016/j.freeradbiomed.2021.12.267.

251. Choi J, Yang ES, Cha K, Whang J, Choi WJ, Avraham S, Kim TA. The nuclear matrix protein, NRP/B, acts as a transcriptional repressor of E2F-mediated transcriptional activity. J Cancer Prev. 2014;19(3):187–98. https://doi.org/10.15430/JCP.2014.19.3.187.

252. Luo S, Lei K, Xiang D, Ye K. NQO1 Is regulated by PTEN in glioblastoma, mediating cell proliferation and oxidative stress. Oxid Med Cell Longev. 2018;2018:9146528. https://doi.org/10.1155/2018/9146528.

253. Lei K, Gu X, Alvarado AG, Du Y, Luo S, Ahn EH, Kang SS, Ji B, Liu X, Mao H, Fu H, Kornblum HI, Jin L, Li H, Ye K. Discovery of a dual inhibitor of NQO1 and GSTP1 for treating glioblastoma. J Hematol Oncol. 2020;13(1):141. https://doi.org/10.1186/s13045-020-00979-y.

254. Deland K, Starr BF, Mercer JS, Byemerwa J, Crabtree DM, Williams NT, Luo L, Ma Y, Chen M, Becher OJ, Kirsch DG. Tumor genotype dictates radiosensitization after Atm deletion in primary brainstem glioma models. J Clin Investig. 2021;131(1): e142158. https://doi.org/10.1172/JCI142158.

255. Yu J, Zhong B, Jin L, Hou Y, Ai N, Ge W, Li L, Liu S, Lu JJ, Chen X. 2-Methoxy-6-acetyl-7-methyljuglone (MAM) induced programmed necrosis in glioblastoma by targeting NAD(P)H: Quinone oxidoreductase 1 (NQO1). Free Radical Biol Med. 2020;152:336–47. https://doi.org/10.1016/j.freeradbiomed.2020.03.026.

256. Li LS, Reddy S, Lin ZH, Liu S, Park H, Chun SG, Bornmann WG, Thibodeaux J, Yan J, Chakrabarti G, Xie XJ, Sumer BD, Boothman DA, Yordy JS. NQO1-mediated tumor-selective lethality and radiosensitization for head and neck cancer. Mol Cancer Ther. 2016;15(7):1757–67. https://doi.org/10.1158/1535-7163.MCT-15-0765.

257. He J, Zhou D, Yan B. Eriocitrin alleviates oxidative stress and inflammatory response in cerebral ischemia reperfusion rats by regulating phosphorylation levels of Nrf2/NQO-1/HO-1/NF-κB p65 proteins. Ann Transl Med. 2020;8(12):757. https://doi.org/10.21037/atm-20-4258.

258. Zhong B, Yu J, Hou Y, Ai N, Ge W, Lu JJ, Chen X. A novel strategy for glioblastoma treatment by induction of noptosis, an NQO1-dependent necrosis. Free Radical Biol Med. 2021;166:104–15. https://doi.org/10.1016/j.freeradbiomed.2021.02.014.

259. Zhang L, Wang H. FTY720 inhibits the Nrf2/ARE pathway in human glioblastoma cell lines and sensitizes glioblastoma cells to temozolomide. Pharmacol Rep. 2017;69(6):1186–93. https://doi.org/10.1016/j.pharep.2017.07.003.

260. Hsu SS, Lin YS, Chen HC, Liang WZ. Involvement of oxidative stress-related apoptosis in chlorpyrifos-induced cytotoxicity and the ameliorating potential of the antioxidant vitamin E in human glioblastoma cells. Environ Toxicol. 2023. https://doi.org/10.1002/tox.23850.

261. Lin L, Cai J, Tan Z, Meng X, Li R, Li Y, Jiang C. Mutant IDH1 enhances temozolomide sensitivity via regulation of the ATM/CHK2 pathway in Glioma. Cancer Res Treat. 2021;53(2):367–77. https://doi.org/10.4143/crt.2020.506.

262. Li K, Ouyang L, He M, Luo M, Cai W, Tu Y, Pi R, Liu A. IDH1 R132H mutation regulates glioma chemosensitivity through Nrf2 pathway. Oncotarget. 2017;8(17):28865–79. https://doi.org/10.18632/oncotarget.15868.

263. Cong ZX, Wang HD, Zhou Y, Wang JW, Pan H, Zhang DD, Zhang L, Zhu L. Temozolomide and irradiation combined treatment-induced Nrf2 activation increases chemoradiation sensitivity in human glioblastoma cells. J Neurooncol. 2014;116(1):41–8. https://doi.org/10.1007/s11060-013-1260-x.

264. Strandback E, Lienhart WD, Hromic-Jahjefendic A, Bourgeois B, Högler A, Waltenstorfer D, Winkler A, Zangger K, Madl T, Gruber K, Macheroux P. A small molecule chaperone rescues the stability and activity of a cancer-associated variant of NAD(P)H:quinone oxidoreductase 1 in vitro. FEBS Lett. 2020;594(3):424–38. https://doi.org/10.1002/1873-3468.13636.

265. Liu W, Du M, Wan H, Yang H, Deng X, Chen Y, Zhang Q. Serpin family A member 1 is an oncogene in glioma and its translation is enhanced by NAD(P)H quinone dehydrogenase 1 through RNA-binding activity. Open Med (Warsaw, Poland). 2022;17(1):1645–54. https://doi.org/10.1515/med-2022-0572.

266. Zhan S, Lu L, Pan SS, Wei XQ, Miao RR, Liu XH, Xue M, Lin XK, Xu HL. Targeting NQO1/GPX4-mediated ferroptosis by plumbagin suppresses in vitro and in vivo glioma growth. Br J Cancer. 2022;127(2):364–76. https://doi.org/10.1038/s41416-022-01800-y.

267. Zheng XJ, Chen WL, Yi J, Li W, Liu JY, Fu WQ, Ren LW, Li S, Ge BB, Yang YH, Zhang YZ, Yang H, Du GH, Wang Y, Wang JH. Apolipoprotein C1 promotes glioblastoma tumorigenesis by reducing KEAP1/NRF2 and CBS-regulated ferroptosis. Acta Pharmacol Sin. 2022;43(11):2977–92. https://doi.org/10.1038/s41401-022-00917-3.

268. Zhang K, Chen D, Ma K, Wu X, Hao H, Jiang S. NAD(P)H: quinone oxidoreductase 1 (NQO1) as a therapeutic and diagnostic target in cancer. J Med Chem. 2018;61(16):6983–7003. https://doi.org/10.1021/acs.jmedchem.8b00124.

269. Dinkova-Kostova AT, Talalay P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. Arch Biochem Biophys. 2010;501(1):116–23. https://doi.org/10.1016/j.abb.2010.03.019.

270. Ma X, Moore ZR, Huang G, Huang X, Boothman DA, Gao J. Nanotechnology-enabled delivery of NQO1 bioactivatable drugs. J Drug Target. 2015;23(7–8):672–80. https://doi.org/10.3109/1061186X.2015.1073296.

271. Yang X, Duan J, Wu L. Research advances in NQO1-responsive prodrugs and nanocarriers for cancer treatment. Future Med Chem. 2022;14(5):363–83. https://doi.org/10.4155/fmc-2021-0289.

272. Salama L, Pastor ER, Stone T, Mousa SA. Emerging nanopharmaceuticals and nanonutraceuticals in cancer management. Biomedicines. 2020;8(9):347. https://doi.org/10.3390/biomedicines8090347.

273. Xie J, Tian S, Zhang H, Feng C, Han Y, Dai H, Yan L. A novel NQO1 enzyme-responsive polyurethane nanocarrier for redox-triggered

intracellular drug release. Biomacromol. 2023;24(5):2225–36. https://doi.org/10.1021/acs.biomac.3c00134.

274. Yang YP, Qi FJ, Qian YP, Bao XZ, Zhang HC, Ma B, Dai F, Zhang SX, Zhou B. Developing push-pull hydroxylphenylpolyenylpyridinium chromophores as ratiometric two-photon fluorescent probes for cellular and intravital imaging of mitochondrial NQO1. Anal Chem. 2021;93(4):2385–93. https://doi.org/10.1021/acs.analchem.0c04279.

275. Li J, Cao Y, Zhang X, An M, Liu Y. The application of nano-drug delivery system with sequential drug release strategies in cancer therapy. Am J Clin Oncol. 2023;46(10):459–73. https://doi.org/10.1097/COC.0000000000001030.

276. Yin H, Huang YH, Best SA, Sutherland KD, Craik DJ, Wang CK. An Integrated molecular grafting approach for the design of Keap1-targeted peptide inhibitors. ACS Chem Biol. 2021;16(7):1276–87. https://doi.org/10.1021/acschembio.1c00388.

277. Kumah EA, Fopa RD, Harati S, Boadu P, Zohoori FV, Pak T. Human and environmental impacts of nanoparticles: a scoping review of the current literature. BMC Public Health. 2023;23(1):1059. https://doi.org/10.1186/s12889-023-15958-4.

278. Parkinson EI, Hergenrother PJ. Deoxynyboquinones as NQO1-activated cancer therapeutics. Acc Chem Res. 2015;48(10):2715–23. https://doi.org/10.1021/acs.accounts.5b00365.

279. Gong Q, Yang F, Hu J, Li T, Wang P, Li X, Zhang X. Rational designed highly sensitive NQO1-activated near-infrared fluorescent probe combined with NQO1 substrates in vivo: an innovative strategy for NQO1-overexpressing cancer theranostics. Eur J Med Chem. 2021;224: 113707. https://doi.org/10.1016/j.ejmech.2021.113707.

280. Han J, Cheng L, Zhu Y, Xu X, Ge C. Covalent-assembly based fluorescent probes for detection of hNQO1 and imaging in living cells. Front Chem. 2020;8:756. https://doi.org/10.3389/fchem.2020.00756.

281. Punganuru SR, Madala HR, Arutla V, Srivenugopal KS. Cancer-specific biomarker hNQO1-activatable fluorescent probe for imaging cancer cells in vitro and in vivo. Cancers. 2018;10(12):470. https://doi.org/10.3390/cancers10120470.

282. Peng B, Chen G, Li Y, Zhang H, Shen J, Hou JT, Li Z. NQO-1 enzyme-activated NIR theranostic agent for pancreatic cancer. Anal Chem. 2022;94(32):11159–67. https://doi.org/10.1021/acs.analchem.2c01189.

283. Zhao Y, Hu Q, Cheng F, Su N, Wang A, Zou Y, Hu H, Chen X, Zhou HM, Huang X, Yang K, Zhu Q, Wang X, Yi J, Zhu L, Qian X, Chen L, Tang Y, Loscalzo J, Yang Y. SoNar, a highly responsive NAD+/NADH sensor, allows high-throughput metabolic screening of anti-tumor agents. Cell Metab. 2015;21(5):777–89. https://doi.org/10.1016/j.cmet.2015.04.009.

284. Ross D, Siegel D. NQO1 in protection against oxidative stress. Curr Opinion Toxicol. 2018;7:67–72. https://doi.org/10.1016/j.cotox.2017.10.005.

Publisher's Note