Molecules and Cells

Minireview

Anti-Ferroptotic Effects of Nrf2: Beyond the Antioxidant Response

Aryatara Shakya[1], Nicholas W. McKee[1], Matthew Dodson[1], Eli Chapman[1], and Donna D. Zhang[1,2,*]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA, [2]The University of Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA
*Correspondence: zhangd@arizona.edu
https://doi.org/10.14348/molcells.2023.0005
www.molcells.org

The transcription factor Nrf2 was originally identified as a master regulator of redox homeostasis, as it governs the expression of a battery of genes involved in mitigating oxidative and electrophilic stress. However, the central role of Nrf2 in dictating multiple facets of the cellular stress response has defined the Nrf2 pathway as a general mediator of cell survival. Recent studies have indicated that Nrf2 regulates the expression of genes controlling ferroptosis, an iron- and lipid peroxidation-dependent form of cell death. While Nrf2 was initially thought to have anti-ferroptotic function primarily through regulation of the antioxidant response, accumulating evidence has indicated that Nrf2 also exerts anti-ferroptotic effects via regulation of key aspects of iron and lipid metabolism. In this review, we will explore the emerging role of Nrf2 in mediating iron homeostasis and lipid peroxidation, where several Nrf2 target genes have been identified that encode critical proteins involved in these pathways. A better understanding of the mechanistic relationship between Nrf2 and ferroptosis, including how genetic and/or pharmacological manipulation of Nrf2 affect the ferroptotic response, should facilitate the development of new therapies that can be used to treat ferroptosis-associated diseases.

Keywords: cancer, ferroptosis, Nrf2

INTRODUCTION

The transcription factor nuclear factor erythroid-2 (NF-E2)-related factor 2 (Nrf2, encoded by the *NFE2L2* gene), which was originally identified as a master regulator of the cellular antioxidant response, is now recognized as a crucial mediator of a wide range of cellular processes (Dodson et al., 2019). Along with its role in mitigating oxidative stress, Nrf2 has been shown to regulate iron homeostasis, xenobiotic metabolism, proteostasis, DNA repair, metabolism of carbohydrates, amino acids, and lipids, as well as mitochondrial function (Cuadrado et al., 2019; Hayes and Dinkova-Kostova, 2014; Yamamoto et al., 2018). Nrf2 is essential for maintaining proper cell function, and loss of Nrf2 results in enhanced sensitivity to cytotoxic xenobiotics and increased risk of cell death.

Cell death is tightly regulated at the molecular level, and its effectors are often closely integrated with other key cellular processes. Our understanding of how cell death is initiated continues to evolve, with 12 different modes of programmed cell death having now been identified (Galluzzi et al., 2018). Ferroptosis is a recently discovered form of regulated cell death that is genetically, biochemically, and morphologically distinct from other modes of cell death. Ferroptosis is driven by the accumulation of free labile iron, and increased lipid peroxidation, which represent the primary hallmarks of ferroptosis (Forcina and Dixon, 2019; Stockwell et al., 2017). Interestingly, Nrf2 regulates the expression of many genes

Received January 2, 2023; revised February 6, 2023; accepted February 9, 2023; published online March 24, 2023

eISSN: 0219-1032

responsible for preventing these hallmarks from occurring. Since ferroptosis is an oxidative form of cell death, Nrf2 was initially believed to exert its anti-ferroptotic effects primarily through regulation of the antioxidant response; however, recent studies from our group and others have implicated new mechanisms of Nrf2 regulation of ferroptosis that extend beyond antioxidant function into key facets of iron and lipid homeostasis. In this review, we will provide an in-depth exploration of how Nrf2 regulates ferroptosis, with an emphasis on its antioxidant-independent functions, and how modulation of Nrf2-mediated ferroptotic death could affect various disease states.

OVERVIEW OF FERROPTOSIS

Coined by Brent Stockwell's group in 2012, the term ferroptosis, as the name indicates, is an iron-dependent form of cell death. The actual origins of ferroptosis date back to 2003, with the discovery of two molecules, erastin and RAS Selective Lethal 3 (RSL3), which caused a non-apoptotic form of cell death in mutant HRAS-expressing foreskin fibroblasts that could ultimately be rescued by lipophilic antioxidants or iron chelating agents (Dolma et al., 2003; Stockwell et al., 2017; Yagoda et al., 2007; Yang and Stockwell, 2008). While erastin inhibits the system xCT cystine/glutamate antiporter, causing depletion of intracellular glutathione (GSH) and inactivation of GPX4 due to loss of this critical cofactor, RSL3 directly binds to and inhibits GPX4 function, preventing cells from effectively reducing lipid peroxides and causing eventual cell death (Dixon et al., 2012; Wolpaw et al., 2011). Consistent with these original observations, many other studies over the years have provided evidence indicating that the core ferroptotic cascade includes two salient features: (1) iron accumulation and (2) increased lipid peroxidation as shown in Fig. 1. Nrf2 regulates the major defense pathways responsible for ensuring that these pro-ferroptotic changes are kept in check, indicating its central role in preventing the initiation of the ferroptosis cascade. In the following sections, we will briefly discuss key aspects of each of these critical drivers of ferroptosis, including how they are directly and indirectly influenced by Nrf2 (Fig. 2, Table 1).

Iron homeostasis

Iron exists in two redox states, ferrous (Fe^{2+}) and ferric (Fe^{3+}). While the constant loss or gain of electrons to switch between two redox states makes iron useful for metabolic reactions, the generation of free radicals due to an excess of the highly reactive Fe^{2+} form is toxic to cells. To prevent iron toxicity, free labile iron in the form of (Fe^{2+}) is controlled by multiple systems at both the systemic and cellular levels to maintain iron homeostasis. Systemic iron homeostasis is regulated by hepcidin, a hormone released from the liver. Iron in the blood in the Fe^{3+} form is bound by transferrin (Knutson, 2017). As shown in Fig. 1, transferrin-bound Fe^{3+} is endocytosed into cells by the transferrin receptor (TFR). Once inside the cell, Fe^{3+} is released from TFR and reduced to Fe^{2+} by STEAP3. Fe^{2+} is then transported from the endosomal compartment to the cytoplasm by divalent metal transporter 1 (DMT1), where it is oxidized back to Fe^{3+} and incorporated into proteins or stored in ferritin cages, with the help of iron chaperones such as poly(RC)-binding protein 1 (PCBP1) (Philpott et al., 2017). Excess Fe^{2+} can be exported out of the cell by ferroportin (FPN1/*SLC40A1*) or contribute to the labile iron pool (LIP). Proper iron homeostasis is critical to cell survival, as the presence of excess Fe^{2+} can lead to iron driven generation of the extremely reactive hydroxyl radical via the Fenton reaction, a key upstream driver of ferroptotic death (Dixon et al., 2012; Kagan et al., 2017; Xie et al., 2016; Yang et al., 2016; Zou et al., 2020). The importance of iron in promoting ferroptosis is further evidenced by the fact that iron chelators block ferroptotic cell death both *in vitro* and *in vivo*, and exogenous iron supplementation sensitizes cells to ferroptosis inducers (i.e., erastin) (Dixon et al., 2012; Hou et al., 2016; Li et al., 2017).

Several Nrf2 target genes have been shown to regulate critical aspects of iron homeostasis, including heme biosynthesis and catabolism, as well as iron uptake, export, storage, and utilization (Fig. 2, Table 1). As a significant portion of functional iron in the body is in the form of heme, dysregulation of heme metabolism could enhance the risk of undergoing ferroptotic death. Many of the Nrf2 target genes involved in heme metabolism were originally identified by Chip-seq analysis of sulforaphane (SF)-treated lymphoblasts, including ATP Binding Cassette Subfamily B Member 6 (*ABCB6*, porphyrin transport and heme synthesis), ferrochelatase (*FECH*, heme synthesis), solute carrier family 48 member 1 (*SLC48A1*, heme transport), alpha-1-microglobulin (*AMBP*, heme binding/degradation), and thromboxane A synthase 1 (*TBXAS1*) (Campbell et al., 2013; Chorley et al., 2012). Heme oxygenase-1 (*HMOX1*), the enzyme that catalyzes the degradation of heme to produce biliverdin, was initially identified as an Nrf2 target gene in curcumin treated renal epithelial cells (Balogun et al., 2003). Treatment of a variety of cancer cell lines with the $I\kappa B\alpha$ inhibitor BAY11-7085 induced ferroptosis in an *HMOX1*-dependent manner (Chang et al., 2018). Upregulation of *HMOX1* was also associated with increased ferroptotic death in the cardiac tissue of sickle cell disease mice (Menon et al., 2022). Conversely, *HMOX1* was shown to be anti-ferroptotic in erastin treated renal proximal tubule cells (Adedoyin et al., 2018), indicating a possible tissue and context-dependent role for *HMOX1* in mediating ferroptosis. Biliverdin reductases A and B (*BLVRA* and *BLVRB*), which convert biliverdin to bilirubin (a strong antioxidant/free radical scavenger) during heme catabolism, were also shown to be Nrf2 target genes, as assessed by ChIP-seq, DNA microarray, and transcriptomic/proteomic analyses (Agyeman et al., 2012; Hirotsu et al., 2012).

Ferritin is responsible for sequestering free iron and preventing it from participating in Fenton reactions. The ferritin cage is comprised of 24 repeating subunits of the ferritin heavy chain (FTH1) and ferritin light chain (FTL), both of which were identified to contain antioxidant response elements (AREs) within their promoter regions (Chorley et al., 2012; Hintze and Theil, 2005; Pietsch et al., 2003; Tsuji et al., 2000; Wasserman and Fahl, 1997). Ferroportin (*SLC40A1*/FPN1), which as mentioned above mediates the export of excess Fe^{2+}, was also identified to contain an ARE, and can be transcriptionally upregulated by Nrf2 in macrophages (Hara-

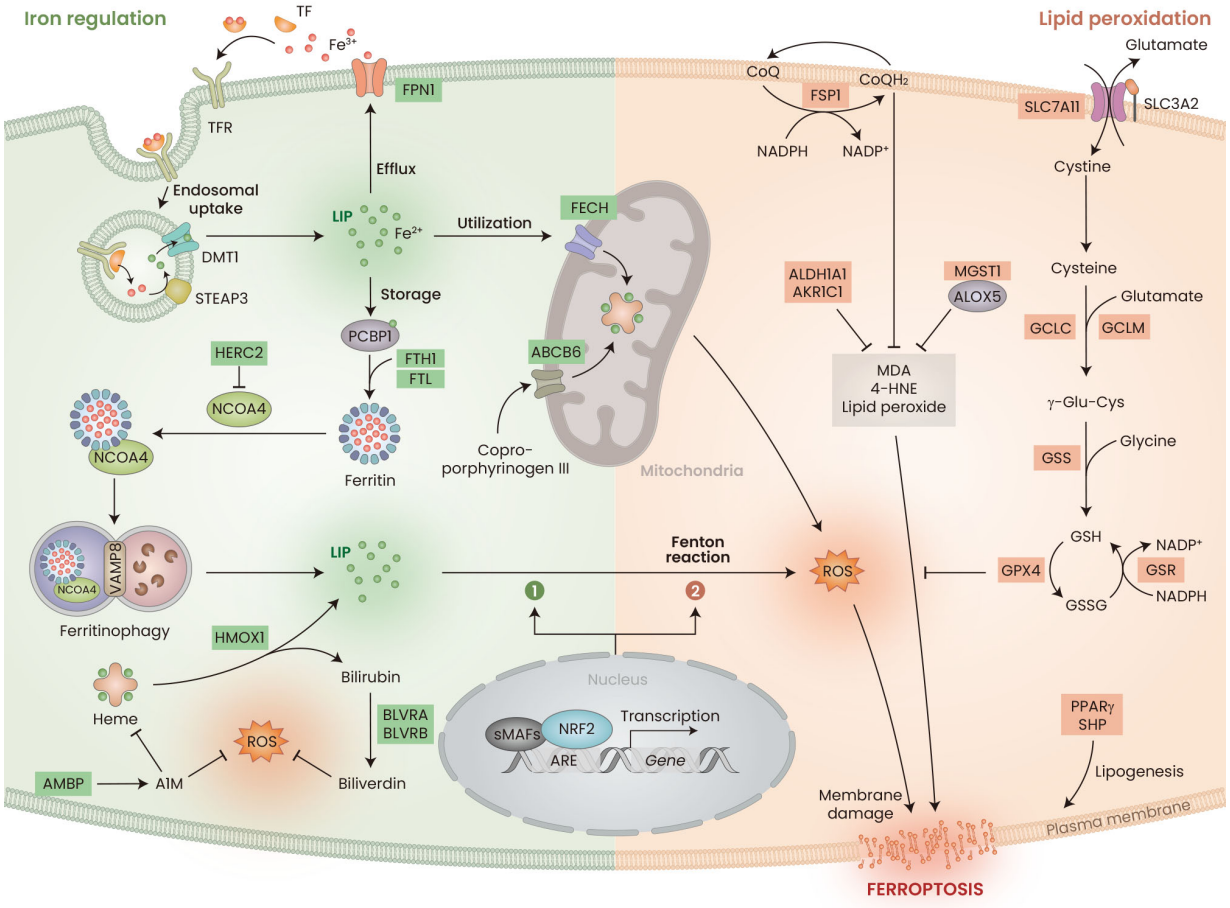

Fig 1. NRF2 target genes regulate both lipid peroxidation and iron homeostasis, critical to ferroptosis. Nrf2 targets controlling iron homeostasis are labeled in green; Nrf2 targets controlling lipid peroxidation are labeled in orange. The core molecular machinery and signaling regulation of ferroptosis can be divided into 1. Iron regulation (left/green) and 2. Lipid peroxidation (right/orange) pathways. 1. Iron Regulation: In the serum, transferrin (TF) binds to ferric iron (Fe^{3+}, red dots) which then encounters the transferrin receptor (TFR) on the cell membrane and is endocytosed into the cell. In the endosome, Fe^{3+} is converted to ferrous iron (Fe^{2+}) by the endosomal STEAP3 metalloreductase. Fe^{2+} is then released into the cytosol via the divalent metal transporter (DMT1), adding to the labile iron pool (LIP). This labile iron can now be exported out of the cell by ferroportin 1 (FPN1), utilized by mitochondria for heme synthesis, or stored in ferritin (FTH1/FTL) via the adapter protein poly(RC)-binding protein 1 (PCBP1). Heme synthesis in the mitochondria is regulated by the ATP binding cassette subfamily B member 6 (*ABCB6*) and ferrochelatase (FECH). ABCB6 transports coproporphyrinogen III from the cytosol to the mitochondrial intermembrane space and Fe^{2+} iron is incorporated into protoporphyrin IX (a metabolite of coproporphyrinogen III) to form heme. For heme degradation, heme-oxygenase 1 (HMOX1) breaks down heme into biliverdin and free Fe^{2+} iron. Biliverdin is further metabolized to a potent antioxidant called bilirubin by biliverdin reductase A and biliverdin reductase B (BLVRA/BLVRB). Next, the proteolytic cleavage of alpha-1-microglobulin/bikunin precursor (AMBP) results in A1M, which exerts it anti-ferroptosis effects through its binding to heme, preventing heme degradation and iron release, as well as inhibiting reactive oxygen species (ROS). When free iron levels are high, iron is stored as Fe^{3+} in ferritin, which can undergo ferritinophagy, i.e., autophagic degradation of ferritin via nuclear receptor coactivator 4 (NCOA4, a cargo-binding protein), which releases iron into the cytosol as Fe^{2+} and contributes to the LIP. HERC2 is an E3 ubiquitin ligase for NCOA4 that promotes its proteasomal degradation thus regulating the ferritin and free iron levels. 2. Lipid peroxidation: The free iron comprising the LIP (Fe^{2+}) is highly redox reactive, which generate ROS via the Fenton reaction. ROS in turn cause lipid peroxidation that damage cell/organelle membranes, generating the reactive lipid species that execute ferroptosis. Production of lipid peroxides and their by-products (MDA, malondialdehyde and 4-HNE, 4-hydroxynonenal) are typically controlled by two critical defense systems, SLC7A11/xCT and GPX4, that are cellular targets for erastin and RSL3, respectively. The amino acid antiporter SLC7A11/xCT facilitates cystine import and glutamate export. Once inside the cell, cystine is reduced to cysteine, and subsequently joined to glutamate and glycine by glutamate-cysteine ligase (GCL, encoded by catalytic and modulatory subunits *GCLC* and *GCLM*, respectively) and glutathione synthetase (GSS) to form glutathione (GSH). GSH is a common cofactor for redox enzymes, such as glutathione peroxidase 4 (GPX4), which converts reactive aldehydes to their alcohol form. The aldehyde dehydrogenase (ALDH1A1) and aldo-keto-reductase (AKR1C1) family of proteins are also associated with reducing lipid peroxides to their less toxic forms. More recently, ferroptosis suppressor protein 1 (FSP1) was found to be a GSH independent ferroptosis suppressor that cooperates with Coenzyme Q10 ($CoQH_2$) to prevent formation of lipid peroxides. Microsomal glutathione S-transferase 1 (*MGST1*) catalyzes the conjugation of GSH to lipid peroxides. Peroxisome proliferator-activated receptor gamma (PPARγ) and small heterodimer partner (SHP) are nuclear receptors involved in lipid metabolism and thereby influence ferroptosis. ARE, antioxidant response elements; GSR, glutathione reductase.

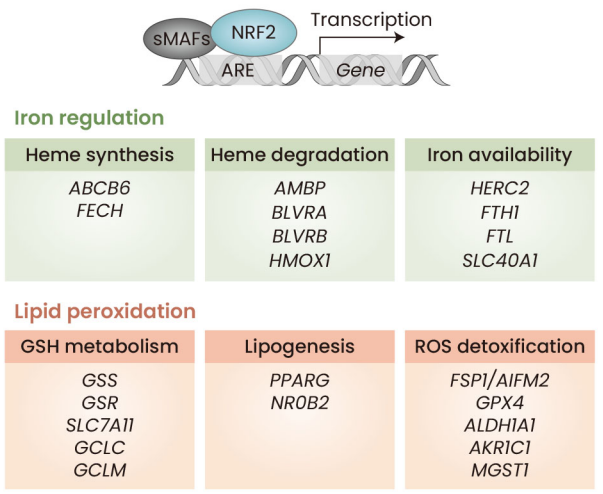

Fig 2. Nrf2 dependent transcriptional regulation of genes controlling ferroptosis. Nrf2 is no longer degraded in the presence of oxidative stress, or as a result of mutations in its E3 ligase complex proteins, most frequently Keap1, enabling its nuclear translocation. In the nucleus, Nrf2 heterodimerizes with sMaf and binds to the antioxidant response element (ARE) containing region to allow the transcription of target genes. As a negative regulator of ferroptosis, Nrf2 regulates the expression of several genes responsible for 1. Iron regulation and 2. Lipid peroxidation. These targets are broadly categorized, including examples for each, into three classes for iron regulation (heme synthesis, heme degradation and iron availability) and three classes for lipid peroxidation (glutathione [GSH] production, lipogenesis, and reactive oxygen species [ROS] detoxification).

da et al., 2011; Marro et al., 2010; Namgaladze et al., 2022), although Nrf2 was also reported to suppress FPN1 in other cell types (Kong et al., 2019). Recently, our group showed that Nrf2 regulates iron homeostasis by controlling both ferritin synthesis and degradation as illustrated in Fig. 3 (Anandhan et al., 2023). First, *HERC2*, encoding an E3 ubiquitin ligase for NCOA4 (a cargo adapter for ferritin degradation by autophagy [ferritinophagy]) and FBXL5 (an iron-dependent regulator of IRP1/2 that binds to the iron responsive element in the 5'-UTR of *FTH1* and *FTL* mRNA thereby repressing their translation and preventing ferritin synthesis) was identified as a novel Nrf2 target gene. Second, Nrf2 indirectly regulates ferritinophagy via TFEB-dependent transcription of the lysosomal SNARE protein VAMP8 (mediates autophagosome-lysosome fusion). Thus, Nrf2 regulates ferritin protein stability at both the translational and ferritinophagy-dependent turnover levels. Critically, we showed that these two mechanisms are vital to mediating Nrf2-driven resistance to ferroptosis induction in cancer cells, as deletion of Nrf2 resulted in ferritinophagy blockage, NCOA4-dependent recruitment of apoferritin to the autophagosome, an elevated LIP, and enhanced sensitivity to the ferroptosis inducer imidazole ketone erastin (Anandhan et al., 2023). Overall, Nrf2 regulation of iron homeostasis is a critical determinant of a cell's sensitivity or resistance to ferroptosis, which is independent of its anti-

Table 1. Putative Nrf2 target genes involved in regulating ferroptosis

Gene symbol	Gene name	ARE verification	Function	Reference
ABCB6	ATP binding cassette subfamily B member 6	ChIP-seq	Mitochondrial uptake of porphyrins.	(Campbell et al., 2013; Chorley et al., 2012)
AKR1C1 *AKR1C2* *AKR1C3*[a]	Aldo-keto reductase family 1 member C1/C2/C3	EMSA RGA ChIP-seq	Belongs to aldo-keto reductase family, reduces aldehydes, ketones, and quinones to corresponding alcohol.	(Burchiel et al., 2007; Hirotsu et al., 2012; Lou et al., 2006; MacLeod et al., 2009)
ALDH1A1	Aldehyde dehydrogenase 1 family member A1	ChIP-seq	Belongs to aldehyde dehydrogenase family, oxidizes aldehydes to carboxylic acid.	(Hirotsu et al., 2012)
AMBP	Alpha-1-microglobulin/bikunin precursor	ChIP-seq	Encodes A1M and bikunin proteins which have roles in heme catabolism and structural incorporation of extracellular matrix.	(Campbell et al., 2013)
BLVRB	Biliverdin reductase A/B	ChIP-seq	NADPH or NADH-dependent catalysis of the conversion of biliverdin to bilirubin.	(Agyeman et al., 2012; Hirotsu et al., 2012)
FECH	Ferrochelatase	ChIP-seq	Catalyzes the installation of ferrous iron into protoporphyrin IX.	(Campbell et al., 2013; Chorley et al., 2012)
FSP1 (*AIFM2*)	Apoptosis inducing factor mitochondria associated 2	RGA ChIP-seq	CoQ oxidoreductase that generates the reduced form of coenzyme Q_{10}, which traps lipid peroxide.	(Chorley et al., 2012; Koppula et al., 2022)

Table 1. Continued

Gene symbol	Gene name	ARE verification	Function	Reference
FTH1	Ferritin heavy chain 1	EMSA RGA ChIP-seq	Heavy chain of ferritin which is a major intracellular iron storage protein; FTH1 is involved in the oxidation of Fe^{2+} to Fe^{3+}.	(Chorley et al., 2012; Pietsch et al., 2003)
FTL	Ferritin light chain	RGA ChIP-seq	Light chain of ferritin which is a major intracellular iron storage protein.	(Chorley et al., 2012; Hintze and Theil, 2005)
GCLC *GCLM*	Glutamate-cysteine ligase catalytic/ modifier subunit	EMSA RGA ChIP-seq	GCLC is the catalytic subunit of glutamate-cysteine ligase (GCS) while GCLM is the modifier subunit of GCS. GCS is the first rate-limiting enzyme of glutathione synthesis.	(Chorley et al., 2012; Erickson et al., 2002; Hirotsu et al., 2012; Yang et al., 2005)
GPX4	Glutathione peroxidase 4	ChIP-seq	Catalyzes the reduction of hydrogen peroxide, organic hydroperoxides, and lipid hydroperoxides.	(Hirotsu et al., 2012)
GSS	Glutathione synthetase	EMSA RGA	Forms homodimer which catalyzes the ATP-dependent conversion of gamma-L-glutamyl-L-cysteine to glutathione.	(Lee et al., 2005)
GSR	Glutathione-disulfide reductase	RGA ChIP-seq	Reduces sulfur atoms in a glutathione disulfide bond (GS-SG) into their sulfhydryl form (GSH).	(Chorley et al., 2012; Harvey et al., 2009; Wang et al., 2007)
HMOX1	Heme oxygenase 1	EMSA RGA ChIP-seq	Cleaves heme to form biliverdin.	(Balogun et al., 2003; Campbell et al., 2013; Chorley et al., 2012; Hirotsu et al., 2012)
MT1	Metallothionein 1	RGA ChIP-seq	Cysteine rich proteins involved in metal detoxification and homeostasis as well as protection against oxidative stress.	(Hirotsu et al., 2012; Houessinon et al., 2016)
NR0B2 (SHP)	Nuclear receptor subfamily 0 group B member 2	RGA ChIP-seq	An orphan nuclear receptor that interacts with retinoid receptors, thyroid hormone receptors, and estrogen receptors, preventing the respective receptor's ligand-dependent transcriptional activation.	(Huang et al., 2010)
PPARG	Peroxisome proliferator activated receptor gamma	EMSA ChIP-seq	Nuclear receptor, functioning as a transcription factor that regulates fatty acid storage and cholesterol metabolism.	(Cho et al., 2010; Chorley et al., 2012)
PRDX6	Peroxiredoxin 6	RGA ChIP-seq	Reduces various peroxides such as short chain organic, phospholipid, and fatty acid hydroperoxides.	(Chorley et al., 2012; Chowdhury et al., 2009; Hirotsu et al., 2012)
SLC40A1	Solute carrier family 40 member 1	RGA	Cell membrane protein involved in export of iron from cells.	(Campbell et al., 2013; Marro et al., 2010)
SLC48A1 (HRG1)	Solute carrier family 48 member 1	ChIP-seq	Cell membrane protein involved in heme transport through endosome membrane, lysosomal membrane, and plasma membrane.	
SLC7A11	Solute carrier family 7 member 11	RGA ChIP-seq	Cell membrane protein that mediates the transport of anionic cysteine in exchange for glutamate.	(Hirotsu et al., 2012; Sasaki et al., 2002)
TBXAS1	Thromboxane A synthase 1	ChIP-seq	Monooxygenase that catalyzes the conversion of prostaglandin H2 to thromboxane A2.	(Campbell et al., 2013)

These genes listed were identified by ChIP-Seq analysis and subsequently validated by the indicated experimental method used to identify a functional antioxidant response elements (AREs), including electrophoretic mobility shift assay (EMSA), reporter gene assay (RGA), or computational search (CS).
[a]AKR1C3: The ARE verification for AKR1C3 was not performed by Chip-seq, EMSA, or RGA.

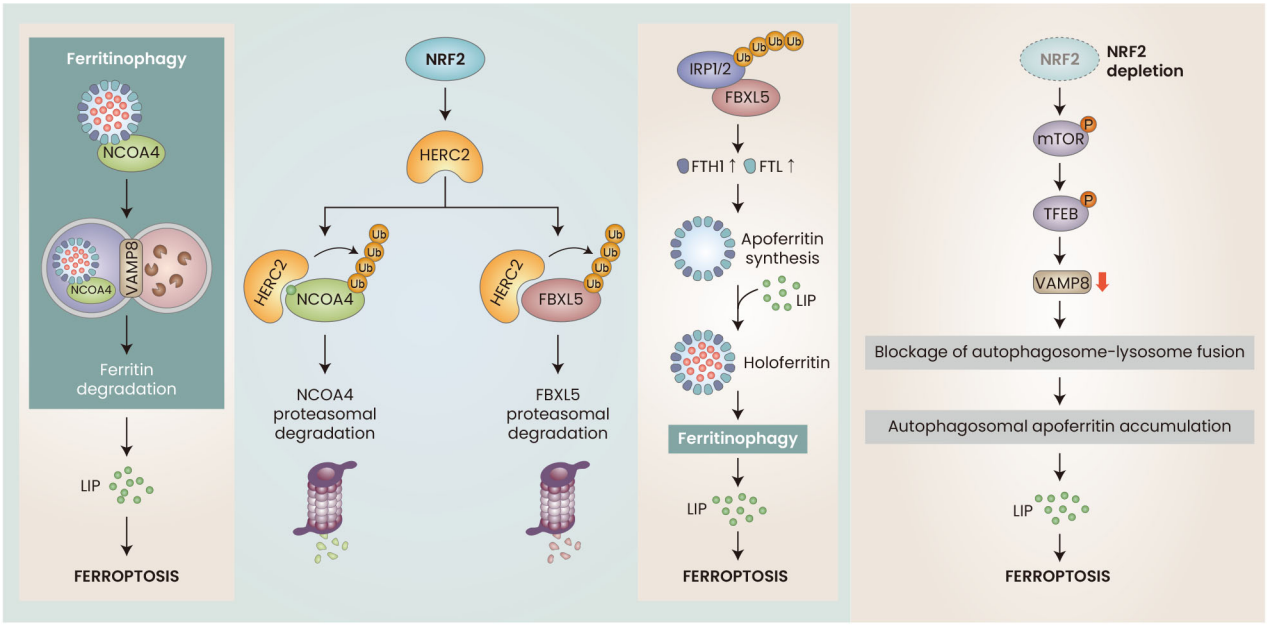

Fig 3. Nrf2 dependent post translational regulation of ferroptosis. Nrf2 regulates ferritin synthesis and degradation at the post translational level via its transcriptional target, *HERC2* and indirect target, *VAMP8*, overall facilitating an anti-ferroptotic effect. HERC2 is an E3 ubiquitin ligase for NCOA4 (ferritin cargo receptor mediating ferritin degradation, i.e. ferritinophagy [box in green]) and FBXL5 (IRP1/2 regulator mediating ferritin synthesis). Presence of Nrf2 results in HERC2 mediated proteasomal degradation of NCOA4 and FBXL5, inhibiting ferroptosis. Loss of Nrf2, on the other hand, results in decreased *VAMP8* expression and NCOA4-dependent ferritinophagy blockage and subsequent accumulation of apoferritin in the autophagosome, leading to less iron storage and an increase in the labile ferrous iron (Fe^{2+}) levels, leading to ferroptosis activation. For detailed information, please review Fig. 4 in our recent paper (Anandhan et al., 2023).

oxidant function, as discussed below.

Lipid peroxidation

Lipid peroxides are formed when polyunsaturated fatty acids (PUFAs) in the cell membrane, or organellar membranes are oxidized by reactive species, including hydroxyl and hydroperoxyl radicals, reactive nitrogen species (i.e., peroxynitrite), and the actual end products of lipid peroxidation themselves (4-HNE [4-hydroxynonenal] and MDA [malondialdehyde]) (Yin et al., 2011). These highly reactive and electrophilic lipid peroxides, which have a variety of cytotoxic consequences, are now recognized along with free iron as key players in promoting the ferroptotic cascade (Ayala et al., 2014; Conrad et al., 2018). Nrf2 governs the expression of a host of target genes responsible for preventing the formation of lipid peroxides (Fig. 2, Table 1). The CoQ oxidoreductase *AIFM2/FSP1* (GSH-independent ferroptosis suppressor), a lipophilic antioxidant that inhibits ferroptosis independently of GPX4, was found to be an Nrf2 target gene (Bersuker et al., 2019; Doll et al., 2019; Emmanuel et al., 2022; Koppula et al., 2022). An earlier study using ChIP seq analysis also identified that Nrf2 could bind to the promoter region of *AIFM2* (now called *FSP1*) in SF-treated lymphoblasts (Chorley et al., 2012). Some members of the aldo-keto-reductase family, i.e., *AKR1C1-3*, *AKR1B1*, and *AKR1B10*, are well-established Nrf2 target genes that have all been associated with ferroptosis resistance, as they convert lipid peroxides into less toxic intermediates (Burchiel et al., 2007; Dixon et al., 2014; Hirotsu et al., 2012; Lou et al., 2006; MacLeod et al., 2009). Similarly, aldehyde dehydrogenase 1 family member A1 (*ALDH1A1*), which catalyzes the oxidation of reactive aldehydes to their less reactive carboxylic acid form, is an Nrf2 target gene (Hirotsu et al., 2012). Peroxisome proliferator-activated receptor gamma (*PPARG*) and nuclear receptor subfamily 0, group B, member 2 (*NR0B2*), which encode PPARγ and small heterodimer partner (SHP), respectively, are nuclear receptors that play essential roles in regulating cholesterol metabolism and thus the structural integrity of lipid membranes. Both were identified as Nrf2 target genes in an *in vivo* comparison of Nrf2 wild type versus KO mice, as well as *in vitro* in Nrf2 induced lymphoblasts (Cho et al., 2010; Chorley et al., 2012; Huang et al., 2010). These studies indicate that Nrf2 directly regulates the expression of several target genes that play a critical role in mitigating the accumulation of lipid peroxides, maintaining membrane integrity, and preventing the subsequent progression of the ferroptotic cascade.

With the production of reactive lipid peroxides that compromise cell membrane integrity and damage DNA, proteins, and organelles, proper processing of these peroxides is critical to prevent their harmful pro-ferroptotic effects. Under ferroptotic conditions, several PUFA metabolizing enzymes, including Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4), Prostaglandin-endoperoxide synthase 2/cyclooxygenase 2 (PTGS2/COX-2), and Arachidonate-5/12/15-li-

poxygenase (ALOX5, ALOX12, and ALOX15) are elevated, all of which are regarded as biomarkers of ferroptotic death (Chen et al., 2021; Chu et al., 2019; Dixon et al., 2015; Doll et al., 2017; Yang et al., 2014). In contrast, two critical proteins that prevent excess lipid peroxidation are glutathione peroxidase 4 (GPX4, reduces lipid peroxides to their alcohol form) and system xc- (cystine/glutamate antiporter), which as mentioned above, were identified as the targets of the first ferroptosis inducers RSL3 and erastin, respectively (Dixon et al., 2012; Wolpaw et al., 2011; Yang and Stockwell, 2008). Accordingly, inhibition of these defense mechanisms plays a significant role in promoting ferroptosis. In fact, the ferroptosis inducers identified to date all target these proteins and can be categorized into four classes: (1) Class 1 – system xc- inhibitors (i.e., erastin and its analogs, sulfasalazine, sorafenib), (2) Class 2 – direct inhibitors of GPX4 (i.e., RSL3, RSL5), (3) Class 3 – depleters of GPX4 protein and CoQ10 (i.e., FIN56), and (4) Class 4 (i.e., $FINO_2$) – indirect inhibitors of GPX4 (Abrams et al., 2016; Conrad et al., 2016; Doll et al., 2017; Shimada et al., 2016; Yang and Stockwell, 2016). Intriguingly, both *GPX4* and *SLC7A11* (subunit of system xc-) have been reported as Nrf2 target genes (Hirotsu et al., 2012; Osburn et al., 2006; Salazar et al., 2006). However, it is important to note that while *SLC7A11* is a verified target gene, data supporting *GPX4* as an Nrf2 target gene are less convincing, and a functional ARE has not been identified. Along with its regulation of SLC7A11, which provides the cysteine needed to produce GSH, Nrf2 also controls the expression of the catalytic and modulatory subunits of glutamate-cysteine ligase (*GCLC/GCLM*), glutathione reductase (*GSR*), and glutathione synthetase (*GSS*), all of which are vital for GSH metabolism (Fig. 1) (Chan and Kwong, 2000; Chorley et al., 2012; Erickson et al., 2002; Harvey et al., 2009; Hirotsu et al., 2012; Ishii et al., 2000; Kwak et al., 2002; Lee et al., 2005; Sasaki et al., 2002; Wang et al., 2007; Yang et al., 2005). As GSH is a critical cofactor for GPX4, Nrf2 regulation of GSH levels represents a critical aspect of preventing lipid peroxides from accumulating.

THE Nrf2-FERROPTOSIS AXIS IN PATHOLOGY

The pathological role of ferroptosis in human diseases, including cancer, neurodegeneration, liver injury, kidney failure, and diabetes, is an emerging area of research. In this section, we will briefly discuss the studies that have implicated Nrf2 regulation of ferroptosis in mediating disease progression and treatment.

Cancer

The ferroptosis field emerged during the search for novel classes of small molecules that were able to kill resistant cancer cells. Therefore, the function of ferroptosis in cancer is well reported (Dolma et al., 2003; Stockwell et al., 2017; Yagoda et al., 2007). Over the years, more and more ferroptosis inducers have been identified, and their use to kill resistant cancer cells has been demonstrated in many preclinical cancer models. As we gain more knowledge of the anti-ferroptotic function of Nrf2, it is obvious to predict that inhibition of Nrf2 will significantly enhance the efficacy of ferroptosis inducers. Furthermore, many cancer cells have Nrf2 constitutively activated, which results in the upregulation of cytoprotective genes that promote tumor progression and protect cancer cells from chemotherapeutics, this is known as the dark side of Nrf2 (Wang et al., 2008). Activation of Nrf2 has been reported to protect against ferroptosis in different cancer models. For example, Nrf2 upregulation as a result of autophagy receptor p62-dependent sequestration of Keap1 reduced the sensitivity of hepatocellular carcinoma cells (HCC) to erastin- and sorafenib-induced ferroptosis (Sun et al., 2016). Another study using head and neck cancer (HNC) cells indicated that Nrf2 is essential for these cells to evade RSL3-induced ferroptosis (Shin et al., 2018). Finally, a 3D cell culture model using a CRISPR-Cas9-based screening approach revealed the importance of Nrf2 hyperactivation in promoting the proliferation and survival of lung tumor spheroid cells (Takahashi et al., 2020).

A host of well-established Nrf2 target genes, including *SLC7A11*, *FTH1/FTL*, *SLC48A1*, *GPX4*, *PRDX6*, *MT1G*, and *HMOX1*, have all been shown to be critical for cancer cell growth and conferring resistance to ferroptosis (Chorley et al., 2012; Chowdhury et al., 2009; Fan et al., 2017; Hassannia et al., 2019; Hirotsu et al., 2012; Houessinon et al., 2016; Na and Surh, 2014; Sun et al., 2016). Additionally, *MGST1*, a redox-sensitive repressor of ferroptosis in pancreatic cancer cells, was also recently identified as an Nrf2 target gene that dictates pancreatic cancer cell sensitivity to ferroptosis inducers (Kuang et al., 2021). This increased reliance upon Nrf2 and its downstream targets to protect against ferroptosis led to the notion that inhibition of Nrf2 might be needed to synergistically increase the therapeutic efficacy of pro-ferroptotic drugs, particularly against Nrf2 overexpressing cancers. This was demonstrated in a study using the alkaloid trigonelline (a potent, but non-specific Nrf2 inhibitor), which enhanced the anticancer activity of erastin and sorafenib in HCC cells (Sun et al., 2016), as well as artesunate sensitivity in HNC cells (Roh et al., 2017). We have shown that brusatol, the first NRF2 inhibitor developed by our group (Ren et al., 2011), enhanced the efficacy of imidazole ketone erastin in promoting ferroptosis and preventing tumor growth in several pre-clinical models of ovarian cancer (Anandhan et al., 2023). Another study indicated that cotreatment with brusatol and erastin was more effective than either single treatment in killing NSCLC cells, which was partly due to Nrf2 regulation of the FOCAD-FAK signaling pathway (Liu et al., 2020). Overall, cotreatment with pro-ferroptotic therapeutics and an Nrf2 inhibitor represents a promising strategy for inducing ferroptosis to kill resistant cancer cells.

Other diseases

The link between Nrf2 and ferroptosis has also been elucidated in the pathogenesis of diseases other than cancer, indicating the possible relevance of targeting this cascade in other pathological contexts. Studies of Alzheimer's disease (AD) and Parkinson's disease (PD) have suggested that ferroptosis could be a main driver of the neuronal cell death that promotes the progression of these neurodegenerative diseases (Morris et al., 2018). For example, our group detected that reduced Nrf2 expression correlated with decline in function

of neural stem cells isolated from rats during a critical middle age period (Corenblum et al., 2016). Overall, the gradual loss of Nrf2 with aging is thought to increase susceptibility to ferroptosis, which has been recently demonstrated to play critical roles in the pathogenesis of PD, AD, multiple sclerosis, and other neurodegenerative diseases (Yan et al., 2021). A recent study from our group demonstrated that *Nrf2*$^{-/-}$ mice exhibit an age-dependent increase in several key markers of ferroptosis in brain regions associated with PD (A et al., 2022). Aside from neurodegenerative diseases, Nrf2-mediated ferroptosis has also been linked to other high-morbidity diseases, such as diabetes, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease. In an *in vitro* model of diabetic nephropathy, knockdown of Nrf2 increased cell vulnerability to ferroptosis under hyperglycemic conditions, and treatment with an Nrf2 inducer prevented ferroptosis induction (Li et al., 2021). Using *in vitro* hepatocytes and an *in vivo* model of high fat diet-induced NAFLD, our group detected enhanced lipid peroxidation and ferroptosis during the course of NAFLD, in part due to downregulation of ATG7-mediated autophagosome biogenesis and reduced Nrf2, which could be partially rescued by pharmacological induction of Nrf2 (Liu et al., 2022). The occurrence of NAFLD is generally associated with increased lipids and triglyceride accumulation arising from an imbalance between lipid acquisition and removal in the hepatocytes, which in turn could lead to more lipid peroxidation. Overall, through this study we showed that a high-fat diet induced NAFLD causes lipid peroxidation and ferroptosis, both of which can be prevented by upregulating Nrf2 as a potential therapeutic strategy. In a cell model of atherosclerosis, qRT-PCR and western blot data showed a positive correlation between serine protease 22 (PRSS2) and Nrf2, which was correlated with decreased ferroptosis sensitivity (Yang et al., 2021). Therefore, in contrast to ferroptosis induction/Nrf2 inhibition in cancer treatment, ferroptosis inhibition/Nrf2 activation represents a more viable therapeutic option in these non-cancer related diseases.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

After almost a decade, it has become evident that Nrf2 plays a key role as a ferroptosis suppressor, which is supported by the fact that many Nrf2 target genes have been demonstrated to play important roles in preventing ferroptosis. This notion is also supported by the fact that two critical drivers of ferroptosis, free labile iron and lipid peroxidation, are regulated by Nrf2. While the bulk of the initial studies investigating Nrf2-mediated ferroptosis primarily focused on its antioxidant functions, its anti-ferroptotic role continues to extend beyond just the antioxidant response with the identification of several novel target genes that regulate critical aspects of iron and lipid homeostasis (Fig. 2, Table 1). Further research is needed to dissect the Nrf2-iron-lipid-ferroptosis axis and its role in different pathological contexts. This remains important to the field, as this pathway represents a therapeutic axis to treat ferroptosis-relevant pathologies, either through Nrf2 inhibition in the case of cancer, or through Nrf2 activation in the case of pathological states involving undesirable ferroptotic cell death. Our understanding of Nrf2-mediated ferroptosis has evolved as a result of recent findings but expanding upon this area of research should enhance the therapeutic potential of Nrf2-ferroptosis-based adjuvant therapies.

ACKNOWLEDGMENTS

D.D.Z. is supported by the following grants from the National Institutes of Health: R35ES031575 and P42ES004940.

AUTHOR CONTRIBUTIONS

A.S., N.W.M., and M.D. wrote the manuscript. A.S. and N.W.M. made the figures and table. D.D.Z. and E.C. edited the final manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ORCID

Aryatara Shakya https://orcid.org/0000-0001-5309-9436
Nicholas W. McKee https://orcid.org/0009-0005-1012-4183
Matthew Dodson https://orcid.org/0000-0002-3743-5476
Eli Chapman https://orcid.org/0000-0002-6310-1664
Donna D. Zhang https://orcid.org/0000-0002-8972-697X

REFERENCES

A, A., W, C., N, N., L, M., M, D., and Zhang, D.D. (2022). alpha-Syn overexpression, NRF2 suppression, and enhanced ferroptosis create a vicious cycle of neuronal loss in Parkinson's disease. Free Radic. Biol. Med. *192*, 130-140.

Abrams, R.P., Carroll, W.L., and Woerpel, K.A. (2016). Five-membered ring peroxide selectively initiates ferroptosis in cancer cells. ACS Chem. Biol. *11*, 1305-1312.

Adedoyin, O., Boddu, R., Traylor, A., Lever, J.M., Bolisetty, S., George, J.F., and Agarwal, A. (2018). Heme oxygenase-1 mitigates ferroptosis in renal proximal tubule cells. Am. J. Physiol. Renal Physiol. *314*, F702-F714.

Agyeman, A.S., Chaerkady, R., Shaw, P.G., Davidson, N.E., Visvanathan, K., Pandey, A., and Kensler, T.W. (2012). Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. Breast Cancer Res. Treat. *132*, 175-187.

Anandhan, A., Dodson, M., Shakya, A., Chen, J., Liu, P., Wei, Y., Tan, H., Wang, Q., Jiang, Z., Yang, K., et al. (2023). NRF2 controls iron homeostasis and ferroptosis through HERC2 and VAMP8. Sci. Adv. *9*, eade9585.

Ayala, A., Munoz, M.F., and Arguelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. *2014*, 360438.

Balogun, E., Foresti, R., Green, C.J., and Motterlini, R. (2003). Changes in temperature modulate heme oxygenase-1 induction by curcumin in renal epithelial cells. Biochem. Biophys. Res. Commun. *308*, 950-955.

Bersuker, K., Hendricks, J., Li, Z., Magtanong, L., Ford, B., Tang, P.H., Roberts, M.A., Tong, B., Maimone, T.J., Zoncu, R., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature *575*, 688-692.

Burchiel, S.W., Thompson, T.A., Lauer, F.T., and Oprea, T.I. (2007). Activation of dioxin response element (DRE)-associated genes by benzo(a)pyrene 3,6-quinone and benzo(a)pyrene 1,6-quinone in MCF-10A human mammary epithelial cells. Toxicol. Appl. Pharmacol. *221*, 203-214.

Campbell, M.R., Karaca, M., Adamski, K.N., Chorley, B.N., Wang, X., and Bell, D.A. (2013). Novel hematopoietic target genes in the NRF2-mediated transcriptional pathway. Oxid. Med. Cell. Longev. *2013*, 120305.

Chan, J.Y. and Kwong, M. (2000). Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein. Biochim. Biophys. Acta *1517*, 19-26.

Chang, L.C., Chiang, S.K., Chen, S.E., Yu, Y.L., Chou, R.H., and Chang, W.C. (2018). Heme oxygenase-1 mediates BAY 11-7085 induced ferroptosis. Cancer Lett. *416*, 124-137.

Chen, X., Li, J., Kang, R., Klionsky, D.J., and Tang, D. (2021). Ferroptosis: machinery and regulation. Autophagy *17*, 2054-2081.

Cho, H.Y., Gladwell, W., Wang, X., Chorley, B., Bell, D., Reddy, S.P., and Kleeberger, S.R. (2010). Nrf2-regulated PPAR{gamma} expression is critical to protection against acute lung injury in mice. Am. J. Respir. Crit. Care Med. *182*, 170-182.

Chorley, B.N., Campbell, M.R., Wang, X., Karaca, M., Sambandan, D., Bangura, F., Xue, P., Pi, J., Kleeberger, S.R., and Bell, D.A. (2012). Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. Nucleic Acids Res. *40*, 7416-7429.

Chowdhury, I., Mo, Y., Gao, L., Kazi, A., Fisher, A.B., and Feinstein, S.I. (2009). Oxidant stress stimulates expression of the human peroxiredoxin 6 gene by a transcriptional mechanism involving an antioxidant response element. Free Radic. Biol. Med. *46*, 146-153.

Chu, B., Kon, N., Chen, D., Li, T., Liu, T., Jiang, L., Song, S., Tavana, O., and Gu, W. (2019). ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. Nat. Cell Biol. *21*, 579-591.

Conrad, M., Angeli, J.P., Vandenabeele, P., and Stockwell, B.R. (2016). Regulated necrosis: disease relevance and therapeutic opportunities. Nat. Rev. Drug Discov. *15*, 348-366.

Conrad, M., Kagan, V.E., Bayir, H., Pagnussat, G.C., Head, B., Traber, M.G., and Stockwell, B.R. (2018). Regulation of lipid peroxidation and ferroptosis in diverse species. Genes Dev. *32*, 602-619.

Corenblum, M.J., Ray, S., Remley, Q.W., Long, M., Harder, B., Zhang, D.D., Barnes, C.A., and Madhavan, L. (2016). Reduced Nrf2 expression mediates the decline in neural stem cell function during a critical middle-age period. Aging Cell *15*, 725-736.

Cuadrado, A., Rojo, A.I., Wells, G., Hayes, J.D., Cousin, S.P., Rumsey, W.L., Attucks, O.C., Franklin, S., Levonen, A.L., Kensler, T.W., et al. (2019). Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat. Rev. Drug Discov. *18*, 295-317.

Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell *149*, 1060-1072.

Dixon, S.J., Patel, D.N., Welsch, M., Skouta, R., Lee, E.D., Hayano, M., Thomas, A.G., Gleason, C.E., Tatonetti, N.P., Slusher, B.S., et al. (2014). Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. Elife *3*, e02523.

Dixon, S.J., Winter, G.E., Musavi, L.S., Lee, E.D., Snijder, B., Rebsamen, M., Superti-Furga, G., and Stockwell, B.R. (2015). Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. ACS Chem. Biol. *10*, 1604-1609.

Dodson, M., de la Vega, M.R., Cholanians, A.B., Schmidlin, C.J., Chapman, E., and Zhang, D.D. (2019). Modulating NRF2 in disease: timing is everything. Annu. Rev. Pharmacol. Toxicol. *59*, 555-575.

Doll, S., Freitas, F.P., Shah, R., Aldrovandi, M., da Silva, M.C., Ingold, I., Grocin, A.G., Xavier da Silva, T.N., Panzilius, E., Scheel, C., et al. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. Nature *575*, 693-698.

Doll, S., Proneth, B., Tyurina, Y.Y., Panzilius, E., Kobayashi, S., Ingold, I., Irmler, M., Beckers, J., Aichler, M., Walch, A., et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat. Chem. Biol. *13*, 91-98.

Dolma, S., Lessnick, S.L., Hahn, W.C., and Stockwell, B.R. (2003). Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. Cancer Cell *3*, 285-296.

Emmanuel, N., Li, H., Chen, J., and Zhang, Y. (2022). FSP1, a novel KEAP1/NRF2 target gene regulating ferroptosis and radioresistance in lung cancers. Oncotarget *13*, 1136-1139.

Erickson, A.M., Nevarea, Z., Gipp, J.J., and Mulcahy, R.T. (2002). Identification of a variant antioxidant response element in the promoter of the human glutamate-cysteine ligase modifier subunit gene. Revision of the ARE consensus sequence. J. Biol. Chem. *277*, 30730-30737.

Fan, Z., Wirth, A.K., Chen, D., Wruck, C.J., Rauh, M., Buchfelder, M., and Savaskan, N. (2017). Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. Oncogenesis *6*, e371.

Forcina, G.C. and Dixon, S.J. (2019). GPX4 at the crossroads of lipid homeostasis and ferroptosis. Proteomics *19*, e1800311.

Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W., et al. (2018). Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. *25*, 486-541.

Harada, N., Kanayama, M., Maruyama, A., Yoshida, A., Tazumi, K., Hosoya, T., Mimura, J., Toki, T., Maher, J.M., Yamamoto, M., et al. (2011). Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. Arch. Biochem. Biophys. *508*, 101-109.

Harvey, C.J., Thimmulappa, R.K., Singh, A., Blake, D.J., Ling, G., Wakabayashi, N., Fujii, J., Myers, A., and Biswal, S. (2009). Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. Free Radic. Biol. Med. *46*, 443-453.

Hassannia, B., Vandenabeele, P., and Vanden Berghe, T. (2019). Targeting ferroptosis to iron out cancer. Cancer Cell *35*, 830-849.

Hayes, J.D. and Dinkova-Kostova, A.T. (2014). The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem. Sci. *39*, 199-218.

Hintze, K.J. and Theil, E.C. (2005). DNA and mRNA elements with complementary responses to hemin, antioxidant inducers, and iron control ferritin-L expression. Proc. Natl. Acad. Sci. U. S. A. *102*, 15048-15052.

Hirotsu, Y., Katsuoka, F., Funayama, R., Nagashima, T., Nishida, Y., Nakayama, K., Engel, J.D., and Yamamoto, M. (2012). Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res. *40*, 10228-10239.

Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M.T., Zeh, H.J., 3rd, Kang, R., and Tang, D. (2016). Autophagy promotes ferroptosis by degradation of ferritin. Autophagy *12*, 1425-1428.

Houessinon, A., Francois, C., Sauzay, C., Louandre, C., Mongelard, G., Godin, C., Bodeau, S., Takahashi, S., Saidak, Z., Gutierrez, L., et al. (2016). Metallothionein-1 as a biomarker of altered redox metabolism in hepatocellular carcinoma cells exposed to sorafenib. Mol. Cancer *15*, 38.

Huang, J., Tabbi-Anneni, I., Gunda, V., and Wang, L. (2010). Transcription factor Nrf2 regulates SHP and lipogenic gene expression in hepatic lipid metabolism. Am. J. Physiol. Gastrointest. Liver Physiol. *299*, G1211-G1221.

Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000). Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J. Biol. Chem. *275*, 16023-16029.

Kagan, V.E., Mao, G., Qu, F., Angeli, J.P., Doll, S., Croix, C.S., Dar, H.H., Liu, B., Tyurin, V.A., Ritov, V.B., et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat. Chem. Biol. *13*, 81-90.

Knutson, M.D. (2017). Iron transport proteins: gateways of cellular and systemic iron homeostasis. J. Biol. Chem. *292*, 12735-12743.

Kong, Y., Hu, L., Lu, K., Wang, Y., Xie, Y., Gao, L., Yang, G., Xie, B., He,

W., Chen, G., et al. (2019). Ferroportin downregulation promotes cell proliferation by modulating the Nrf2-miR-17-5p axis in multiple myeloma. Cell Death Dis. *10*, 624.

Koppula, P., Lei, G., Zhang, Y., Yan, Y., Mao, C., Kondiparthi, L., Shi, J., Liu, X., Horbath, A., Das, M., et al. (2022). A targetable CoQ-FSP1 axis drives ferroptosis- and radiation-resistance in KEAP1 inactive lung cancers. Nat. Commun. *13*, 2206.

Kuang, F., Liu, J., Xie, Y., Tang, D., and Kang, R. (2021). MGST1 is a redox-sensitive repressor of ferroptosis in pancreatic cancer cells. Cell Chem. Biol. *28*, 765-775.e5.

Kwak, M.K., Itoh, K., Yamamoto, M., and Kensler, T.W. (2002). Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol. Cell. Biol. *22*, 2883-2892.

Lee, T.D., Yang, H., Whang, J., and Lu, S.C. (2005). Cloning and characterization of the human glutathione synthetase 5'-flanking region. Biochem. J. *390*, 521-528.

Li, Q., Han, X., Lan, X., Gao, Y., Wan, J., Durham, F., Cheng, T., Yang, J., Wang, Z., Jiang, C., et al. (2017). Inhibition of neuronal ferroptosis protects hemorrhagic brain. JCI Insight *2*, e90777.

Li, S., Zheng, L., Zhang, J., Liu, X., and Wu, Z. (2021). Inhibition of ferroptosis by up-regulating Nrf2 delayed the progression of diabetic nephropathy. Free Radic. Biol. Med. *162*, 435-449.

Liu, P., Anandhan, A., Chen, J., Shakya, A., Dodson, M., Ooi, A., Chapman, E., White, E., Garcia, J.G., and Zhang, D.D. (2022). Decreased autophagosome biogenesis, reduced NRF2, and enhanced ferroptotic cell death are underlying molecular mechanisms of non-alcoholic fatty liver disease. Redox Biol. *59*, 102570.

Liu, P., Wu, D., Duan, J., Xiao, H., Zhou, Y., Zhao, L., and Feng, Y. (2020). NRF2 regulates the sensitivity of human NSCLC cells to cystine deprivation-induced ferroptosis via FOCAD-FAK signaling pathway. Redox Biol. *37*, 101702.

Lou, H., Du, S., Ji, Q., and Stolz, A. (2006). Induction of AKR1C2 by phase II inducers: identification of a distal consensus antioxidant response element regulated by NRF2. Mol. Pharmacol. *69*, 1662-1672.

MacLeod, A.K., McMahon, M., Plummer, S.M., Higgins, L.G., Penning, T.M., Igarashi, K., and Hayes, J.D. (2009). Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. Carcinogenesis *30*, 1571-1580.

Marro, S., Chiabrando, D., Messana, E., Stolte, J., Turco, E., Tolosano, E., and Muckenthaler, M.U. (2010). Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. Haematologica *95*, 1261-1268.

Menon, A.V., Liu, J., Tsai, H.P., Zeng, L., Yang, S., Asnani, A., and Kim, J. (2022). Excess heme upregulates heme oxygenase 1 and promotes cardiac ferroptosis in mice with sickle cell disease. Blood *139*, 936-941.

Morris, G., Berk, M., Carvalho, A.F., Maes, M., Walker, A.J., and Puri, B.K. (2018). Why should neuroscientists worry about iron? The emerging role of ferroptosis in the pathophysiology of neuroprogressive diseases. Behav. Brain Res. *341*, 154-175.

Na, H.K. and Surh, Y.J. (2014). Oncogenic potential of Nrf2 and its principal target protein heme oxygenase-1. Free Radic. Biol. Med. *67*, 353-365.

Namgaladze, D., Fuhrmann, D.C., and Brune, B. (2022). Interplay of Nrf2 and BACH1 in inducing ferroportin expression and enhancing resistance of human macrophages towards ferroptosis. Cell Death Discov. *8*, 327.

Osburn, W.O., Wakabayashi, N., Misra, V., Nilles, T., Biswal, S., Trush, M.A., and Kensler, T.W. (2006). Nrf2 regulates an adaptive response protecting against oxidative damage following diquat-mediated formation of superoxide anion. Arch. Biochem. Biophys. *454*, 7-15.

Philpott, C.C., Ryu, M.S., Frey, A., and Patel, S. (2017). Cytosolic iron chaperones: proteins delivering iron cofactors in the cytosol of mammalian cells. J. Biol. Chem. *292*, 12764-12771.

Pietsch, E.C., Chan, J.Y., Torti, F.M., and Torti, S.V. (2003). Nrf2 mediates the induction of ferritin H in response to xenobiotics and cancer chemopreventive dithiolethiones. J. Biol. Chem. *278*, 2361-2369.

Ren, D., Villeneuve, N.F., Jiang, T., Wu, T., Lau, A., Toppin, H.A., and Zhang, D.D. (2011). Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. Proc. Natl. Acad. Sci. U. S. A. *108*, 1433-1438.

Roh, J.L., Kim, E.H., Jang, H., and Shin, D. (2017). Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. Redox Biol. *11*, 254-262.

Salazar, M., Rojo, A.I., Velasco, D., de Sagarra, R.M., and Cuadrado, A. (2006). Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J. Biol. Chem. *281*, 14841-14851.

Sasaki, H., Sato, H., Kuriyama-Matsumura, K., Sato, K., Maebara, K., Wang, H., Tamba, M., Itoh, K., Yamamoto, M., and Bannai, S. (2002). Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. J. Biol. Chem. *277*, 44765-44771.

Shimada, K., Skouta, R., Kaplan, A., Yang, W.S., Hayano, M., Dixon, S.J., Brown, L.M., Valenzuela, C.A., Wolpaw, A.J., and Stockwell, B.R. (2016). Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. Nat. Chem. Biol. *12*, 497-503.

Shin, D., Kim, E.H., Lee, J., and Roh, J.L. (2018). Nrf2 inhibition reverses resistance to GPX4 inhibitor-induced ferroptosis in head and neck cancer. Free Radic. Biol. Med. *129*, 454-462.

Stockwell, B.R., Friedmann Angeli, J.P., Bayir, H., Bush, A.I., Conrad, M., Dixon, S.J., Fulda, S., Gascon, S., Hatzios, S.K., Kagan, V.E., et al. (2017). Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell *171*, 273-285.

Sun, X., Ou, Z., Chen, R., Niu, X., Chen, D., Kang, R., and Tang, D. (2016). Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology *63*, 173-184.

Takahashi, N., Cho, P., Selfors, L.M., Kuiken, H.J., Kaul, R., Fujiwara, T., Harris, I.S., Zhang, T., Gygi, S.P., and Brugge, J.S. (2020). 3D culture models with CRISPR screens reveal hyperactive NRF2 as a prerequisite for spheroid formation via regulation of proliferation and ferroptosis. Mol. Cell *80*, 828-844.e6.

Tsuji, Y., Ayaki, H., Whitman, S.P., Morrow, C.S., Torti, S.V., and Torti, F.M. (2000). Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. Mol. Cell. Biol. *20*, 5818-5827.

Wang, X., Tomso, D.J., Chorley, B.N., Cho, H.Y., Cheung, V.G., Kleeberger, S.R., and Bell, D.A. (2007). Identification of polymorphic antioxidant response elements in the human genome. Hum. Mol. Genet. *16*, 1188-1200.

Wang, X.J., Sun, Z., Villeneuve, N.F., Zhang, S., Zhao, F., Li, Y., Chen, W., Yi, X., Zheng, W., Wondrak, G.T., et al. (2008). Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis *29*, 1235-1243.

Wasserman, W.W. and Fahl, W.E. (1997). Functional antioxidant responsive elements. Proc. Natl. Acad. Sci. U. S. A. *94*, 5361-5366.

Wolpaw, A.J., Shimada, K., Skouta, R., Welsch, M.E., Akavia, U.D., Pe'er, D., Shaik, F., Bulinski, J.C., and Stockwell, B.R. (2011). Modulatory profiling identifies mechanisms of small molecule-induced cell death. Proc. Natl. Acad. Sci. U. S. A. *108*, E771-E780.

Xie, Y., Hou, W., Song, X., Yu, Y., Huang, J., Sun, X., Kang, R., and Tang, D. (2016). Ferroptosis: process and function. Cell Death Differ. *23*, 369-379.

Yagoda, N., von Rechenberg, M., Zaganjor, E., Bauer, A.J., Yang, W.S., Fridman, D.J., Wolpaw, A.J., Smukste, I., Peltier, J.M., Boniface, J.J., et al.

(2007). RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature *447*, 864-868.

Yamamoto, M., Kensler, T.W., and Motohashi, H. (2018). The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol. Rev. *98*, 1169-1203.

Yan, H.F., Zou, T., Tuo, Q.Z., Xu, S., Li, H., Belaidi, A.A., and Lei, P. (2021). Ferroptosis: mechanisms and links with diseases. Signal Transduct. Target. Ther. *6*, 49.

Yang, H., Magilnick, N., Lee, C., Kalmaz, D., Ou, X., Chan, J.Y., and Lu, S.C. (2005). Nrf1 and Nrf2 regulate rat glutamate-cysteine ligase catalytic subunit transcription indirectly via NF-kappaB and AP-1. Mol. Cell. Biol. *25*, 5933-5946.

Yang, K., Song, H., and Yin, D. (2021). PDSS2 inhibits the ferroptosis of vascular endothelial cells in atherosclerosis by activating Nrf2. J. Cardiovasc. Pharmacol. *77*, 767-776.

Yang, W.S., Kim, K.J., Gaschler, M.M., Patel, M., Shchepinov, M.S., and Stockwell, B.R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proc. Natl. Acad. Sci. U. S. A. *113*, E4966-E4975.

Yang, W.S., SriRamaratnam, R., Welsch, M.E., Shimada, K., Skouta, R., Viswanathan, V.S., Cheah, J.H., Clemons, P.A., Shamji, A.F., Clish, C.B., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. Cell *156*, 317-331.

Yang, W.S. and Stockwell, B.R. (2008). Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. Chem. Biol. *15*, 234-245.

Yang, W.S. and Stockwell, B.R. (2016). Ferroptosis: death by lipid peroxidation. Trends Cell Biol. *26*, 165-176.

Yin, H., Xu, L., and Porter, N.A. (2011). Free radical lipid peroxidation: mechanisms and analysis. Chem. Rev. *111*, 5944-5972.

Zou, Y., Henry, W.S., Ricq, E.L., Graham, E.T., Phadnis, V.V., Maretich, P., Paradkar, S., Boehnke, N., Deik, A.A., Reinhardt, F., et al. (2020). Plasticity of ether lipids promotes ferroptosis susceptibility and evasion. Nature *585*, 603-608.