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REVIEW ARTICLE

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p53 in ferroptosis regulation: the new weapon for the old guardian

Yanqing Liu[1] and Wei Gu[1,2]✉

Although the conventional activities of p53 such as cell cycle arrest, senescence, and apoptosis are well accepted as the major checkpoints in stress responses, accumulating evidence implicates the importance of other tumor suppression mechanisms. Among these unconventional activities, an iron-dependent form of non-apoptotic cell death, termed ferroptosis, attracts great interest. Unlike apoptotic cell death, activation of p53 alone is not sufficient to induce ferroptosis directly; instead, through its metabolic targets, p53 is able to modulate the ferroptosis response in the presence of ferroptosis inducers such as GPX4 inhibitors or high levels of ROS. Here, we review the role of ferroptosis in p53-mediated tumor suppression, with a focus on what cellular factors are critical for p53-dependent ferroptosis during tumor suppression and how p53 modulates both the canonical (GPX4-dependent) and the non-canonical (GPX4-independent) ferroptosis pathways. We also discuss the possibility of targeting p53-mediated ferroptotic responses for the treatment of human cancers and potentially, other diseases.

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FACTS

1. p53 has a crucial but complex role in regulating ferroptosis.
2. Ferroptosis acts as an independent pathway for suppressing tumor growth and is tightly connected with metabolism and oxidative stress responses.
3. p53-mediated ferroptosis is required for its tumor suppression in mouse models.
4. A number of ferroptosis inducers have been proposed as potential drugs for cancer therapy.

OPEN QUESTIONS

1. What is the physiological ferroptosis inducer(s) for p53 during tumor suppression?
2. Why does p53 either promote or repress ferroptosis under different biological settings?
3. Is p53-dependent ferroptosis sufficient for tumor suppression in the absence of cell cycle arrest, senescence, and apoptosis?
4. Can ferroptosis be specifically induced in tumors but not in normal tissues?

INTRODUCTION

The tumor suppressor TP53 (also called p53) has been among the most extensively studied genes since its discovery in 1979 [1, 2]. Over millions of years of evolution, p53 has been highly conserved across species, thus suggesting its critical roles [3]. In humans, p53 has been called the "guardian of the genome" or "guardian of the cell" because of its roles in responding to various internal or external stresses, including DNA damage, oncogene activation, dysregulated metabolism, ribosomal stress, and telomere erosion [3–5]. Once activated, p53 coordinates multiple downstream pathways, thereby maintaining the homeostasis of the host cell or organism (if the stress is mild, transient, and repairable) or eliminating damaged cells (if the stress is acute, prolonged, and difficult to resist). To achieve this, p53 mainly functions as a transcription factor (TF) targeting hundreds of genes, but it also has TF-independent roles in the nucleus and cytoplasm [6].

Most studies in the p53 field have focused on its roles in tumors. In the first decade since its discovery, p53 was considered an oncogene. However, vast data from 30 years of further study indicate a tumor-suppressive role of p53 [1, 3]. Among them, the following three genetic findings strongly support p53 as an important tumor suppressor: (1) the p53 gene is mutated in more than 50% of human tumors; (2) people with Li–Fraumeni syndrome (who carry an inherited mutant p53 allele) are highly predisposed to a wide range of tumor types; (3) p53 null mice develop tumors with 100% penetrance. p53's ability to inhibit tumors can be considered as a specific outcome of its

[1]Institute for Cancer Genetics, and Herbert Irving Comprehensive Cancer Center, Vagelos College of Physicians & Surgeons, Columbia University, 1130 Nicholas Ave, New York, NY 10032, USA. [2]Department of Pathology and Cell Biology, Vagelos College of Physicians & Surgeons, Columbia University, 1130 Nicholas Ave, New York, NY 10032, USA.
✉email: wg8@cumc.columbia.edu

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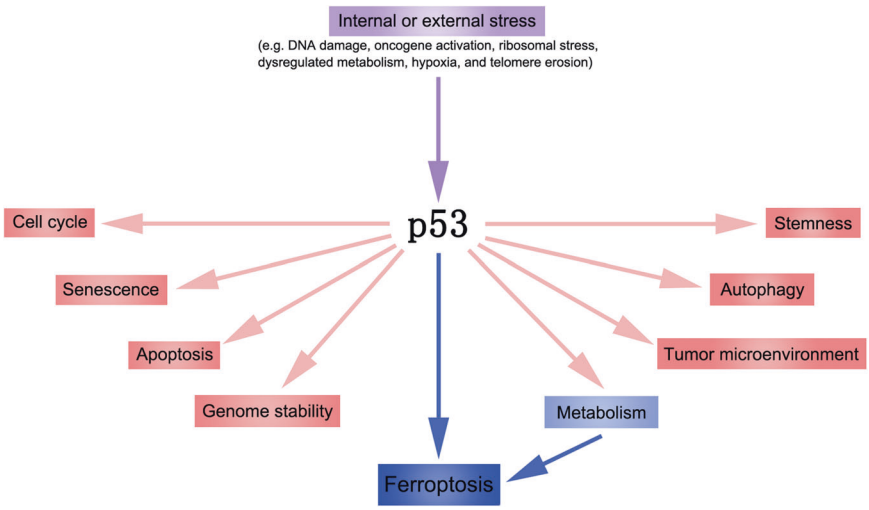

Fig. 1 Major functions of p53. Various stresses can activate p53 to exert diverse functions, including the classical functions (inducing cell cycle arrest, senescence, apoptosis, maintaining genome stability, modulating metabolism, and autophagy. p53 also influences the tumor microenvironment) and the emerging novel functions (regulating ferroptosis and stemness). All these functions contribute to the tumor-suppressive effect of p53.

stress-responsive effects. The initiation and development of tumor cell is accompanied by elevated stresses, such as oncogene activation, DNA damage, genome instability, and reprogrammed tumor metabolism. When responding to these stresses, p53 may incidentally but efficiently suppress tumors. To date, various mechanisms have been suggested to explain the powerful tumor-suppressive effect of p53, including the induction of cell cycle arrest, senescence, and apoptosis. However, the debate regarding which single function of p53 is absolutely critical for its tumor suppressor role is ongoing [7].

Ferroptosis, a term coined in 2012 [8, 9], is a newly identified type of regulated cell death (RCD). Indeed, ferroptosis has gained substantial attention in both basic research and clinical applications. Numerous cellular factors and potential regulatory pathways underlying ferroptosis have been elucidated (details below). Ferroptosis is associated with a variety of physiological or pathological processes, such as normal development, ischemic injuries, degenerative diseases, and immune system activities, and remarkably is also involved in tumor biology [10, 11]. The physiopathological relevance of ferroptosis makes it an attractive therapeutic target to cure different diseases. Since the first research linking p53 to ferroptosis regulation was reported in 2015 [12], more than 170 studies have been published on p53 and ferroptosis. In this review, we briefly discuss the paradigm shift in the understanding of how p53 suppresses tumors and describe the basic biology underlying ferroptosis. We then focus on how p53 mediates ferroptosis and how this pathway might be regulated and targeted to treat diverse diseases, particularly tumors.

CLASSICAL TUMOR-SUPPRESSIVE FUNCTIONS OF P53

After decades of intensive study, many modes of action for p53 in tumor inhibition have been proposed, including promoting cell cycle arrest, senescence, and apoptosis—three typical effects of p53's response to DNA damage (Box 1); regulating cancer cell metabolism and stemness; and mediating ferroptosis [13] (Fig. 1). p53 is not likely to rely on a single path of action but instead probably uses a well-orchestrated network of mechanisms to restrict tumor development. However, deciphering the specific contribution of each mechanism and determining the underlying hierarchy among them remains important.

In 2011–2013, three independent studies challenged the necessity of the DNA damage response effects (induction of cell cycle arrest, senescence, and apoptosis) in p53-mediated tumor inhibition [14–16]. Laura Attardi's laboratory generated different p53 transactivation domain (TAD) mutation knockin mouse strains [14]. After mutation of two critical amino acids in TAD1 (L25Q;W26S), p53 loses its ability to transactivate most of its target genes, such as p21, Puma, and Noxa. This mutant does not respond to acute DNA damage by causing cell cycle arrest and apoptosis. However, the $p53^{25,26}$ mutant has been found to efficiently decrease the tumor burden in a $Kras^{G12D}$-driven lung cancer mouse model. These data suggest that cell cycle arrest and apoptosis are dispensable for p53-mediated tumor repression in certain contexts. The tumor-suppressive activity of the $p53^{25,26}$ mutant might be due to a remaining ability to induce cell senescence or other functions. Acetylation is a crucial post-translational modification determining p53 activity [5]. Therefore, our laboratory designed a $p53^{3KR}$ knockin mouse by mutating three critical lysine residues (K117, K161, and K162) to arginines (K117R, K161R, and K162R) [15]. These mutations abrogate p53's ability to transcriptionally induce targets involved in cell cycle arrest (p21 and GADD45), senescence (p21 and Pml), and apoptosis (Puma, Noxa, Bax, and Dr5). However, we observed that compared with p53 null mice, which developed spontaneous tumors quickly after birth, most of the $p53^{3KR}$ mice remained healthy until at least 16 months of age. This result clearly indicated that p53 suppresses tumor growth in the absence of typical DNA damage response activities. In addition, Andreas Strasser's laboratory has demonstrated that $p21^{-/-}Puma^{-/-}Noxa^{-/-}$ mice are not predisposed to spontaneous tumors, thus further deemphasizing the role of the DNA damage response effects in p53's tumor-suppressive activity. These three studies collectively support an argument against the dogma that the induction of cell cycle arrest, senescence, and apoptosis is absolutely required for p53's function as a tumor suppressor.

Then what are the exact functions contributing to p53's tumor-suppressive effect? Further investigation of $p53^{3KR}$ mice in our laboratory revealed that this mutant retains the ability to mediate cell metabolism [15], which is another key function of p53 beyond the DNA damage response [17]. In fact, p53 is involved in the regulation of a plethora of metabolic pathways. Importantly, most of the p53-mediated metabolic activities antagonize the vast and rewired metabolic demands of tumor cells [17]. Therefore, p53 might reasonably be expected to use its metabolism-regulatory

ability (beyond its DNA damage response effects) to suppress tumor growth. Even so, one question at the operational level remains: what is the exact activity that p53 uses to eradicate tumor cells by interfering with their metabolism?

CANONICAL FERROPTOSIS MODEL

Ferroptosis is defined as "a form of RCD initiated by oxidative perturbations of the intracellular microenvironment that is under constitutive control by GPX4 and can be inhibited by iron chelators and lipophilic antioxidants," according to the Nomenclature Committee on Cell Death, 2018 [18]. Before this concept was formulated, many research clues hinted at the existence of this form of RCD [9]. In 2003, aiming to identify small molecules selectively targeting and killing mutant HRas-bearing human oncogenic fibroblast cells, Brent Stockwell's laboratory performed a high-throughput screening of thousands of chemical compounds [19]. The researchers successfully identified a small molecule erastin that sensitizes cells to a non-apoptotic cell death process. Later, they identified another compound, RSL3, that specifically killed RAS mutant cells [20]. During their exploration of the molecular mechanisms of cell death induced by Erastin and RSL3, a novel cell death modality emerged, which was ultimately termed ferroptosis [8]. Subsequently, many studies on the mechanism and clinical application of ferroptosis have been published. Searching PubMed with the keyword "ferroptosis" returns about 3000 articles published after 2012. The principal features of ferroptosis are now known, and were initially concluded by Stockwell to comprise three hallmarks: "the loss of lipid peroxide repair capacity by the phospholipid hydroperoxidase GPX4, the availability of redox-active iron, and oxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids" [21]. However, as studies in this field go deeper and wider, new results regarding the characteristics of ferroptosis are emerging beyond this old model. To reconcile all these data, the features of ferroptosis can be reduced to three basic elements: substrate of lipid peroxidation, executor of lipid peroxidation, and anti-ferroptosis system (Fig. 2 and Box 2). Any molecular change or pharmacological intervention that regulates any of these elements may affect the final consequences of ferroptosis.

P53 AS A MASTER REGULATOR OF FERROPTOSIS

Ferroptosis is caused by dysregulated cell metabolism (including iron, lipid, amino acids, and ROS metabolism). A major function of p53 is mediating cellular and systematic metabolism. Interestingly, p53 is tightly associated with all key metabolic pathways involved in ferroptosis [17]. Logically, p53 would be expected to regulate ferroptosis. The first evidence that p53 influences ferroptosis was reported in 2015 [22]. To date, many more studies have been published that consolidate the notion that p53 is a key regulator of both canonical and non-canonical ferroptosis pathways (Fig. 3).

P53 IN CANONICAL FERROPTOSIS

In the first study investigating the role of p53 in ferroptosis and tumor suppression, we identified SLC7A11 as a direct target gene suppressed by p53 [22]. SLC7A11 is a key component of the cystine-glutamate antiporter (the xCT system), which mediates cellular uptake of extracellular cystine in exchange for intracellular glutamate. Perturbed cystine absorbance decreases downstream GSH biosynthesis, thus diminishing GPX4's ability to antagonize ferroptosis. Interestingly, the aforementioned DNA-damage response-deficient $p53^{3KR}$ also effectively represses SLC7A11, thus inducing ferroptosis, in agreement with the observation that $p53^{3KR}$ mice do not develop tumors early after birth [15]. In a xenograft mouse tumor model, $p53^{3KR}$ has been found to efficiently inhibit tumor growth, which is restored by the overexpression of SLC7A11. These results clearly demonstrate the importance of SLC7A11 inhibition in p53-mediated tumor suppression. In the same study, ferroptosis facilitated by $p53^{3KR}$ has also been found to be associated with mouse embryonic developmental abnormalities and cell sensitivity to ROS. However, $p53^{4KR}$ (K98R+3KR) loses the ability to suppress SLC7A11, thus impairing ferroptosis induction and tumor suppression [23]. These data again support the importance of acetylation in p53's regulatory functions and the role of SLC7A11 inhibition and ferroptosis induction in p53-mediated tumor suppression. Moreover, on the X-ray repair cross-complementing 4 (XRCC4) knockout background, $p53^{3KR}$ mice exhibit clear premature aging, thus underscoring that combined ferroptosis and genomic instability may significantly contribute to aging [24]. Of note, an intact TAD is necessary for p53 to regulate SLC7A11 and ferroptosis, because the $p53^{25,26,53,54}$ mutant cannot repress SLC7A11 and promote ferroptosis [25]. In addition, the p53 P47S polymorphism, commonly found in people of African descent, is also defective in promoting ferroptosis and repressing tumor development [26–28]. Mechanistically, p53 P47S increases the cellular levels of CoA and GSH, thus limiting ferroptosis [27]. The augmented GSH levels may be due to the impaired ability of p53 P47S to downregulate

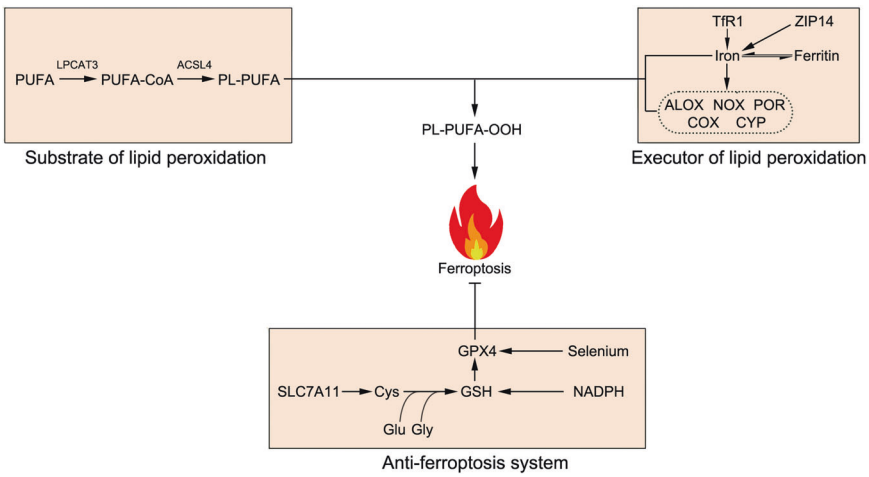

Fig. 2 The canonical ferroptosis pathway. There are three basic elements for ferroptosis: substrate of lipid peroxidation, executor of lipid peroxidation, and anti-ferroptosis system. Disruption of the equilibrium of these elements may lead to ferroptosis. Arrows indicate positive effects. Perpendicular bars indicate negative effects.

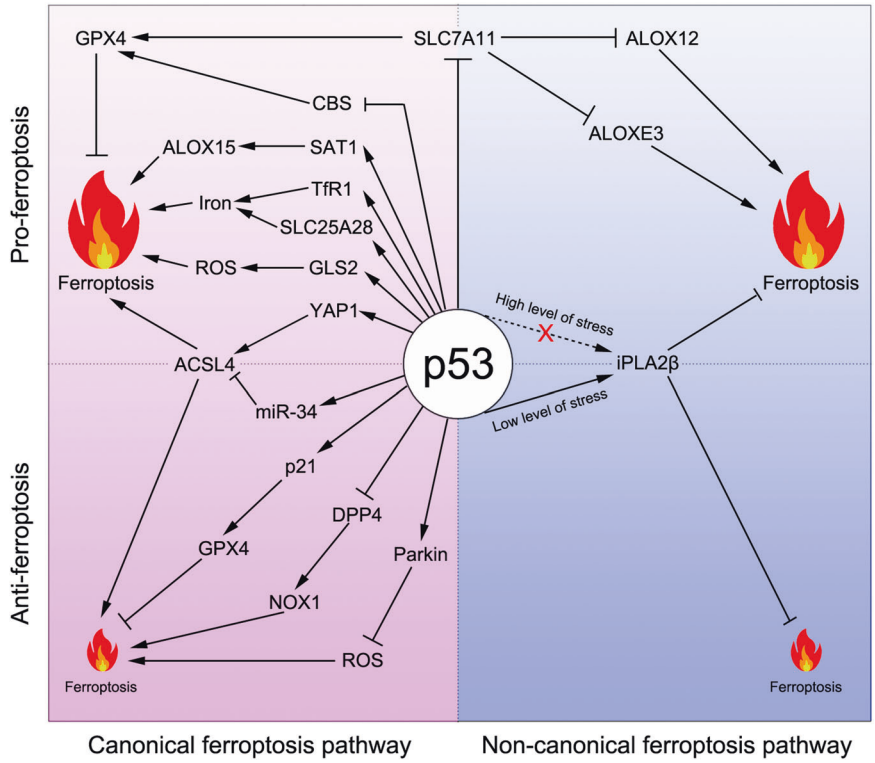

Fig. 3 p53 regulates both canonical and non-canonical ferroptosis pathways. p53 is a master regulator of both canonical and non-canonical ferroptosis pathways via a variety of mechanisms. In most cases, p53 promotes ferroptosis. However, under a certain context, p53 can inhibit ferroptosis. Arrows indicate positive effects. Perpendicular bars indicate negative effects.

SLC7A11 [26]. Moreover, p53 P47S is associated with alterations in the expression or activity of several redox-related proteins, including activating transcription factor 4, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and pyruvate kinase isoform M2, which together produce a ferroptosis-resistant metabolic state in cells [29]. The compromised tumor-suppressive ability of p53 P47S has been found to correlate with an increased risk of breast cancer among premenopausal African-American women [30]. Another study has reported the TF activity-independent mechanism of p53-mediated SLC7A11 inhibition [31]. Monoubiquitinated histone H2B lysine 120 (H2Bub1) is an epigenetic mark indicating active transcription of host genes. After Erastin treatment, H2Bub1 at the SLC7A11 gene decreases, thus resulting in a decreased SLC7A11 protein level. The mechanism is that p53 recruits deubiquitinase ubiquitin-specific peptidase 7 (USP7, or HAUSP) to the promoter region of SLC7A11, which deubiquitinates H2Bub1. This study adds a new regulatory layer to the p53-SLC7A11 axis.

Beyond downregulating SLC7A11 and impairing GSH biogenesis, p53 promotes ferroptosis through regulating other metabolic pathways. Spermidine/spermine N1-acetyltransferase 1 (SAT1) is a rate-limiting enzyme in polyamine catabolism. We have found that p53 transactivates SAT1, thereby slowing xenograft tumor growth [32]. However, SAT1 overexpression does not affect the cell cycle and apoptosis. Interestingly, we have uncovered that SAT1 induction leads to lipid peroxidation and ferroptosis. This effect is due to ALOX15 upregulation after SAT1 induction. Therefore, the p53/SAT1/ALOX15 axis partially contributes to p53-mediated ferroptosis and tumor suppression. Glutaminolysis can drive ferroptosis [33]. p53 also facilitates glutaminolysis, thereby boosting ferroptosis by activating glutaminase 2, a mitochondrial enzyme catalyzing the first step of glutamine catabolism [34]. In addition, in hepatic stellate cells (HSCs), p53 translocates to the mitochondria, where it enhances the activity of SLC25A28, a protein that causes abnormal accumulation of redox-active iron and promotes ferroptosis [35]. Ferredoxin reductase, another p53 target, modulates RSL3- and Erastin-induced ferroptosis, although the exact effect and mechanism remain unclear [36]. In anti-ferroptosis systems, although p53 suppresses SLC7A11 expression, it can also inhibit the serine synthesis pathway and transsulfuration pathway by repressing phosphoglycerate dehydrogenase and cystathionine β-synthase (CBS), respectively, thus limiting GSH production [37, 38]. Mouse double minute 2 homolog (MDM2) is the major E3 ubiquitin-protein ligase that degrades p53, but it is also a p53 target gene. One study has shown that MDM2 and its homolog MDMX amplify ferroptosis through peroxisome proliferator-activated receptor alpha–mediated lipid remodeling and ferroptosis suppressor protein 1 (FSP1) inhibition [39]. Although this effect of MDM2 is p53 independent, given that p53 can transactivate MDM2, p53 may regulate ferroptosis partly via the induction of MDM2. Moreover, p53 has been found to regulate the expression of a ferroptosis marker, prostaglandin-endoperoxide synthase 2 (PTGS2, or COX2) [40, 41]. In summary, p53 promotes ferroptosis through its multipotent roles in regulating cellular metabolism, particularly lipid, iron, ROS, and amino acid metabolism [17]. Whether other metabolic target genes of p53 or other metabolic processes modulated by p53 (such as autophagy) might contribute to the ferroptosis-regulatory role of p53 remains to be clarified.

Most studies on p53 and ferroptosis to date support a ferroptosis-promoting function of p53. However, in certain contexts, p53 may retard or inhibit ferroptosis. In a hyperlipidemia-associated vascular calcification model, p53 has intriguingly been found to elevate SLC7A11 expression, thereby protecting vascular smooth muscle cells (VSMCs) against ferroptotic cell death [42]. Periostin renders VSMCs more sensitive to ferroptosis by inhibiting p53, and this effect is abrogated by metformin treatment. Tarangelo et al. found that pretreating cells

with the MDM2 inhibitor Nutlin-3 to stabilize p53 delays the onset of ferroptosis in cancer cells [43]. This effect relies on the induction of p21 by p53. However, p21-mediated cell cycle arrest itself is not the cause of this delay. In fact, p21 induction redistributes the serine usage from nucleotide biogenesis to GSH synthesis, and GSH is an inhibitor of ROS and ferroptosis [43, 44]. Another study has shown that p21 levels are inversely correlated with cellular sensitivity to ferroptosis inducted by the ferroptosis inducers (FINs) Erastin or IKE [45]. p21 even acts as an independent barrier to ferroptosis in the absence of p53 [45, 46]. In colorectal cancer cell lines SW48 and HCT116, p53 deletion increases the sensitivity to Erastin-triggered ferroptosis [47]. Mechanistically, dipeptidyl peptidase 4 (DPP4) boosts ferroptosis in p53-deficient cells by binding NADPH oxidase 1 and enhancing the generation of ROS leading to lipid peroxidation and ferroptosis. p53 directly binds and sequesters DPP4 in the nucleus. The nuclear retention of DPP4 abolishes its ferroptosis-promoting activity. The mitochondrial activity contributes to ferroptosis [48]. Parkinson disease 2 (PARK2, or Parkin) is a p53 target gene that mediates mitophagy, a process that eliminates damaged mitochondria [49]. This activity decreases the number of mitochondria and the cellular sensitivity to Erastin-mediated, but not RSL3-induced, ferroptosis [48]. Therefore, p53 may limit cysteine deprivation-induced ferroptosis by activating Parkin expression. However, there is also a study reporting an inhibitory effect of p53 on Parkin activity [50]. ACSL4 is a key regulator of membrane PUFA generation for ferroptosis [51]. miR-34, a p53-activated microRNA (miRNA) [52–54], post-transcriptionally downregulates ACSL4 levels [55–57]. The p53/miR-34/ACSL4 axis may repress ferroptosis by limiting the lipid peroxidation substrate. Interestingly, miR-34 induction partly contributes to p53-promoted apoptosis. Therefore, miR-34 may act as a functional switch that dictates the final outcome of p53 activation (causing ferroptosis or apoptosis). Nevertheless, other studies have claimed that p53 upregulates ACSL4 levels [58, 59]. The exact roles of Parkin and ACSL4 in p53-mediated ferroptosis pathway need more investigation.

How can p53 both promote and suppress ferroptosis? Careful examination of these controversial results suggests at least three explanations. The first explanation is the different cell types used in the studies. Different cell types have quite distinct genomic structures, gene expression profiles, and signaling pathways, which together dictate cell behavior after p53 induction. Moreover, the effect of p53 is highly context dependent [60], thus potentially explaining why p53 promotes SLC7A11 expression in VSMCs [42] but suppresses SLC7A11 expression in most other cells. p21 is differentially regulated across cell types and may be the mechanism underlying the differences in sensitivity of these cells to ferroptosis [43, 45]. Ferroptosis is a complex process affected by many factors. Therefore, systemic analysis of the cellular context is helpful to understand the specific contributions of these associated pathways and of each factor, such as p53 [61]. The second explanation is the duality of p53 functions: p53 often has dual roles in regulating activities, depending on the specific cellular state. For example, p53 can both promote and inhibit autophagy, ROS production, and nucleotide synthesis, even in the same cell type [17]. The selectivity of target genes and the effects of p53 are central questions in the p53 field, and both partly depend on the nature of the stress faced by the host cell [17]. The third possible explanation is the different interventions used to induce ferroptosis across experiments. In most studies, researchers have used small molecules, such as Erastin, IKE, and RSL3, as FINs to trigger ferroptosis. This decision is understandable, given that the targets of these FINs are major players in the classical ferroptosis model [21]. However, as this field develops, various exceptional situations not included in the original model are being discovered. For example, p53 has been reported to play essential roles in some non-canonical ferroptosis pathways, in which classical FINs have only minimal effects (described in the next section). The cellular conditions elicited by these molecules may not provide the ideal environment for p53 function; hence, p53 might not fully exert its regulatory functions in these contexts. In this light, using classical FINs to investigate the role of p53 in ferroptosis modulation might be not appropriate and could cause misleading results, as discussed above.

P53 IN NON-CANONICAL FERROPTOSIS

By screening the ALOX arachidonate lipoxygenase family to identify potential contributors to p53-mediated ferroptosis and tumor suppression, we found that ALOX12 is a critical candidate for these functions [62]. Mechanistically, p53 promotes the activity of ALOX12 via inhibiting SLC7A11. SLC7A11 binds and sequesters ALOX12 from its substrate, PUFAs, including those esterified in membranes. When p53 downregulates SLC7A11, ALOX12 is released and subsequently oxidizes membrane PUFAs and initiates ferroptosis. Hence, the p53/SLC7A11/ALOX12 axis is independent of the decrease in GSH biogenesis and GPX4 activity, and therefore is a mechanism distinct from the p53/SLC7A11/GPX4 pathway. Importantly, in the same study, we confirmed that ACSL4 is dispensable for p53/ALOX12-mediated ferroptosis; hence, this axis may be a new pathway outside the classic ferroptosis model. Strikingly, human p53 gene and ALOX12 gene reside in a close genomic site on chromosome 17p13.1, in which gene deletion is a common incident. Maybe these two genes are co-deleted in some tumor types, conferring more survival advantage to these tumors than deleting or inactivating p53 alone [63]. In fact, according to our data, even loss of one allele of ALOX12 will foster the tumor growth while p53 gene is intact [62]. These data underscore the importance of ALOX12 in p53-mediated ferroptosis and tumor suppression. In a recent study, Yang et al. reported that another ALOX family member, ALOXE3, acts in a similar manner to ALOX12 in glioblastoma (GBM) in inducing ferroptosis [64]. SLC7A11 also binds and sequesters ALOXE3 from its substrate, 12-hydroperoxyeicosatetraenoic acids, which is an ALOX12 catalytic product. The inhibition of ALOXE3 increases the production and secretion of 12-hydroxyeicosatetraenoic acids, thus rendering GBM cells less sensitive to p53-dependent ferroptosis and more migratory. Again, p53/SLC7A11/ALOXE3-mediated ferroptosis is independent of ACSL4. Given that ALOXE3 functions downstream of ALOX12, the p53/SLC7A11/ALOX12 and p53/SLC7A11/ALOXE3 axes can function both cooperatively and independently in modulating ferroptosis and tumor suppression.

According to the original model by Stockwell, GPX4 was considered the core factor antagonizing ferroptosis [21]. Other anti-ferroptosis mechanisms independent of GPX4 have since been identified, including the NADPH-FSP1-CoQ_{10} [65, 66], GTP cyclohydrolase 1 (GCH1)-tetrahydrobiopterin (BH4)-CoQ_{10} [67, 68], nitric oxide synthase 2 (iNOS) [69], and endosomal sorting complexes required for transport (ESCRT) [70] systems. However, the ESCRT system lacks specificity for ferroptosis. The iNOS system mainly functions as an immunological protection method. Moreover, the NADPH-FSP1-CoQ_{10} and GCH1-BH4-CoQ_{10} systems share mechanistic similarities with the classic GSH-GPX4 system by reducing peroxidated lipids, thereby avoiding propagation of lipid peroxidation. Is there any type of widely present, highly specific anti-ferroptosis system that acts in a novel, distinct mechanism from those of the GPX4, FSP1, and GCH1 pathways? Because p53 regulates an independent ferroptosis-inducing pathway as the classic one, might it regulate a specific anti-ferroptosis mechanism? Logically, another way to address the lipid peroxidation might exist, through cleaving peroxidated PUFAs from the membrane phospholipids, thus generating free radicals, which in turn can be eliminated by various cellular antioxidant systems [71].

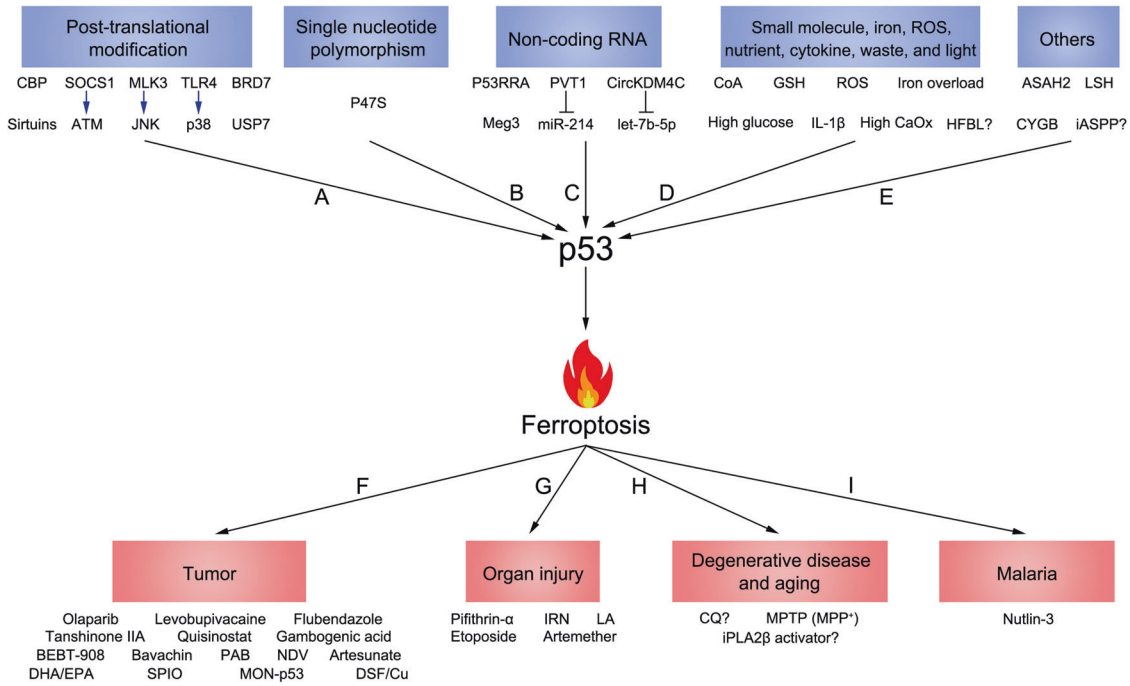

Fig. 4 The complexity of p53-mediated ferroptosis. p53-mediated ferroptosis can be regulated in diverse ways, including **A** post-translational modifications; **B** single-nucleotide polymorphism; **C** non-coding RNAs; **D** small molecules, ROS, iron, nutrient, cytokine, waste, and light; and **E** other proteins. Currently, lots of drugs are demonstrated to affect p53-ferroptosis pathway in distinct disorders, including **F** tumors; **G** organ injuries; **H** degenerative diseases and ageing; and **I** malaria. Blue arrows indicate positive effects. Perpendicular bars indicate negative effects. For the full names of the regulators and drugs, please refer to the main context or Tables 1 and 2.

Recently, our laboratory has identified a novel anti-ferroptosis mechanism acting in this way [72]. Phospholipase A2 group VI (PLA2G6, iPLA2β) is a calcium-independent phospholipase that cleaves acyl tails from the glycerol backbone of lipids and releases oxidized fatty acids [73], which can be further detoxified by the antioxidants in the cytoplasm. We have demonstrated that iPLA2β-mediated detoxification of peroxidated membrane lipids is sufficient to suppress p53/ALOX12-driven ferroptosis in both a GPX4- and an FSP1-independent manner. Interestingly, iPLA2β itself is a p53 target gene that can be activated by p53 after low dose doxorubicin or short term Nutlin treatments. However, increasing the doxorubicin dose or Nutlin treatment time abrogates this activation. This regulation of iPLA2β by p53 has crucial biological significance, because this regulatory axis functions as a stress response process different from the GPX4-mediated constitutive anti-ferroptosis mechanism. GPX4's primary function might be to detoxify basal lipid peroxidation and maintain homeostasis, whereas the p53-iPLA2β axis reacts to the abnormal radical disturbance. The dynamic feature of this regulation also reflects the complexity of p53 functions: when the lipid peroxidation insult is low and consequently can be repaired, p53 transactivates iPLA2β, thus suppressing ferroptosis; in contrast, when this damage persists, or its magnitude is too strong to be restored, p53 induces ferroptosis, which eliminates the damaged cells. This duality of p53 in ferroptosis regulation is reminiscent of how p53 functions in the DNA damage response, cellular metabolism regulation, or non-ferroptosis redox control [17, 74]. Fascinatingly, besides their important roles in tumor, both p53 and iPLA2β are tightly associated with neurodegenerative diseases, including Parkinson's disease (PD) [75, 76]. Ferroptosis has been demonstrated to be a critical pathological factor for neurodegenerative diseases [10, 11, 77]. Therefore, targeting p53/iPLA2β/ferroptosis pathway may bring particular benefits in treating neurodegenerative disease. In summary, this p53-iPLA2β axis works together with other anti-ferroptosis systems to protect cells from excessive ferroptotic cell death and ensure the proper execution of physiologically beneficial ferroptosis.

THE REGULATION OF P53-MEDIATED FERROPTOSIS

p53's activity in ferroptosis, like its other functions, is finely regulated, mainly through modulation of the p53 protein level or activity (Fig. 4A–E and Table 1). Post-translational modification is a critical determinant of p53 function (Fig. 4A). Among the various modification types, acetylation plays a crucial role in dictating the effects of p53 activation [5]. Acetylation not only influences the classic DNA damage response of p53, but also affects its activity in ferroptosis [23, 78]. In 2016, our laboratory found that CREB binding protein acetylates human p53 at K101 (equivalent to mouse p53 K98) [23]. This acetylation is critical for p53-mediated repression of SLC7A11. Mouse $p53^{3KR}$ mutant (K117R, K161R, and K162R) loses the classic DNA damage response but can still inhibit SLC7A11 and suppress tumor growth [15]. However, the mouse $p53^{4KR}$ mutant (K98R, K117R, K161R, and K162R) cannot suppress SLC7A11 and induce ferroptosis. Consistently, mouse $p53^{4KR}$ does not inhibit xenograft tumor growth. The ablated tumor-suppressive function of mouse $p53^{4KR}$ has been further consolidated in a $p53^{4KR}$ knockin mouse model [79]. Compared with $p53^{3KR}$ mice, $p53^{4KR}$ mice develop and succumb to tumors much earlier because of the loss of the ability to promote ferroptosis. Sirtuins are a family of deacetylases closely associated with p53 function [80]. In a myocardial ischemia–reperfusion injury model, p53-activated ferroptosis has been found to contribute to the death of cardiomyocytes [81]. Sirtuin 1 (SIRT1), which is stabilized by USP22, deacetylates p53 and consequently suppresses its inhibitory effect on SLC7A11, thereby protecting cardiomyocytes against ferrotptotic cell death. Similarly, in a traumatic brain injury model, SIRT2 has been found to have a neuroprotective role by inhibiting p53-induced ferroptosis [82]. The mechanism is that the deacetylation of p53 by SIRT2 upregulates SLC7A11 and GPX4

Table 1. Key factors in p53-mediated ferroptosis.

Category of regulator	Name of regulator	Promote (+) or inhibit (–) p53-ferroptosis pathway	Mechanism of action	Physiopathological relevance	Reference
Post-translational protein modification	CREB binding protein (CBP)	+	Acetylate p53 at K101 to boost its suppressive effect on SLC7A11	Promote the tumor-suppressive function of p53	[23, 79]
	Sirtuins	–	Deacetylate p53 to block its suppressive effect on SLC7A11	(1) Protect cardiomyocyte (SIRT1) and neural cell (SIRT2) from ischemia/reperfusion-induced cell death (2) Inhibit the tumor-suppressive function of p53 (SIRT3)	[81–83]
	Suppressor of cytokine signaling 1 (SOCS1)	+	Activate ataxia telangiectasia mutated (ATM) to phosphorylate p53 at S15 and stabilize p53 by interfering tripartite motif containing 28 (TRIM28 or KAP1)	Promote the tumor-suppressive function of p53	[84]
	Mitogen-activated protein kinase kinase kinase 11 (MLK3)	+	Activate mitogen-activated protein kinase 8 (JNK) to phosphorylate and stabilize p53	Contribute to pressure overload-induced myocardial fibrosis	[85]
	Toll-like receptor 4 (TLR4)	+	Activate p38 to phosphorylate p53 to enhance its activity	Contribute to hypoxic-ischemic brain damage (HIBD)	[88]
	Bromodomain containing 7 (BRD7)	+	Promote p53 S392 phosphorylation and mitochondrial localization	Promote ferroptosis in hepatic stellate cells (HSCs) to ameliorate the damage of liver fibrosis	[35]
	Deubiquitinase ubiquitin-specific peptidase 7 (USP7, or HAUSP)	+	Deubiquitinate and stabilize p53 to upregulate transferrin receptor 1 (TfR1)	Contribute to ischemia/reperfusion-induced myocardial injury	[90]
Single-nucleotide polymorphism (SNP)	p53 P47S variant	–	Decrease the ability of p53 to inhibit SLC7A11, increase the cellular levels of CoA and GSH, and produce a ferroptosis-resistant metabolic state in cells	Decrease the ability of p53 to suppress tumor and *Plasmodium* infection, but enhance the response to the malarial toxin hemozoin	[26–30]
Non-coding RNA	P53RRA	+	Bind G3BP stress granule assembly factor 1 (G3BP1) to abolish its repressive effect on p53	Promote the tumor-suppressive function of p53 in breast and lung cancers	[91]
	Meg3	+	Upregulate p53 level to suppress GPX4	Contribute to oxygen and glucose deprivation combined with hyperglycemia-induced diabetic brain ischemic injury	[92]
	PVT1	+	Sponge miR-214 to abolish its repressive effect on p53	Contribute to ischemia/reperfusion-induced brain injury	[93]
	CircKDM4C	+	Sponge hsa-let-7b-5p to abolish its repressive effect on p53	Promote the tumor-suppressive function of p53 in acute myeloid leukemia	[58]
Small molecule, ROS, iron, nutrient, cytokine, waste, and light	CoA	–	Inhibit the oligomerization and activity of p53	Enhance the cellular resistance to ferroptosis	[27]
	GSH				
	ROS	+	Activate the p53/SLC7A11 pathway	Promote the tumor-suppressive function of p53 in lung cancer	[97]
	Iron overload	+	Activate the p53/SLC7A11 pathway	Contribute to sarcopenia, Parkinson's disease, and ischemia-induced hippocampal neuronal death	[98–100]

Table 1. continued

Category of regulator	Name of regulator	Promote (+) or inhibit (–) p53-ferroptosis pathway	Mechanism of action	Physiopathological relevance	Reference
	High glucose	+	Activate the p53/SLC7A11 pathway	Contribute to diabetes-induced endothelial dysfunction	[101]
	Interleukin-1β (IL-1β)				
	High calcium oxalate (CaOx)	+	Increase the levels of p53, ACSL4, transferrin, and TfR	Contribute to the renal crystal deposition and the development of urolithiasis	[102]
	High fluence of blue light (HFBL)	+	Activate p53	May be associated with light pollution and may be used to treat p53-ferroptosis pathway-related diseases	[103]
Others	N-acylsphingosine amidohydrolase (ASAH2)	–	Destabilize p53	Decrease the ability of p53 to suppress tumor by promoting the accumulation of myeloid-derived suppressor cells (MDSCs) in colon cancer microenvironment	[95]
	Lymphoid-specific helicase (LSH)	–	Inactivate p53	Decrease the ability of p53 to suppress tumor	[38]
	Cytoglobin (CYGB)	+	Activate p53/YAP1/ACSL4 pathway	Promote the tumor-suppressive function of p53 in colon cancer	[59]

levels, thus blocking the ferroptosis of neural cells. In addition, we have found that SIRT3 is necessary for repressing p53-mediated ferroptosis in several tumor cells [83], although whether this finding is based on the deacetylation of p53 by SIRT3 remains elusive. Beyond acetylation, phosphorylation is important for p53 activity. Suppressor of cytokine signaling 1 (SOCS1) promotes p53-activated ferroptosis by reinforcing ataxia telangiectasia mutated (ATM)-mediated p53 S15 phosphorylation [84]. Moreover, SOCS1 may also stabilize p53 by interfering with p53 repressor tripartite motif containing 28 (TRIM28, or KAP1). Both activities augment p53's transcriptional function, downregulate SLC7A11, upregulate SAT1, and activate ferroptosis. Mitogen-activated protein kinase 8 (JNK) can stabilize p53 by phosphorylating it to abolish the MDM2-mediated p53 degradation [85]. In cardiomyocyte, pressure overload increases p53 level via a mitogen-activated protein kinase kinase kinase 11 (MLK3)/JNK/p53 axis [86]. The upregulated p53 contributes to MLK3-induced ferroptosis and myocardial fibrosis. p38 can also phosphorylate and activate p53 [87]. After hypoxic-ischemic stress, the expression of Toll-like receptor 4 (TLR4) is increased in mouse hippocampal neuronal cell, which activates a p38/p53/SLC7A11/GPX4 cascade to induce ferroptosis and hypoxic-ischemic brain damage (HIBD) [88]. Inhibitors of TLR4 and p38 effectively block ferroptosis and ameliorate HIBD in a neonatal rat model. In HSCs, treatment with Erastin and RSL3 upregulates bromodomain containing 7 (BRD7) [35]. BRD7 promotes p53 S392 phosphorylation and subsequently p53 mitochondrial localization. In mitochondria, p53 directly binds the mitochondrial iron transporter SLC25A28, thus leading to aberrant accumulation of mitochondrial iron, perturbation of electron transport chain activity, increased ROS production, and finally ferroptosis of HSCs, which ameliorates the damage of liver fibrosis. USP7 acts as a p53 activator by deubiquitinating and stabilizing p53 [89]. In a rat heart ischemia/reperfusion model, USP7 has been found to activate p53, thereby upregulating transferrin receptor 1 (TfR1), and resulting in myocardial cell ferroptosis and heart injury [90].

Several non-coding RNAs (including miRNAs and long non-coding RNAs (lncRNAs)) are involved in the regulation of the p53-associated ferroptosis pathway (Fig. 4C). G3BP stress granule assembly factor 1 (G3BP1) is a repressor of p53. In breast and lung cancers, the lncRNA P53RRA interacts with G3BP1 in the cytoplasm and abolishes this repressive effect [91]. The released p53 accumulates in the nucleus and subsequently causes ferroptosis and apoptosis. In a diabetic brain stroke model, rat brain microvascular endothelial cells undergo ferroptosis after treatment with oxygen and glucose deprivation combined with hyperglycemia [92]. This is due to the upregulation of an lncRNA Meg3, which elevates p53 level to suppress GPX4 activity. In another brain ischemia/reperfusion study, upregulation of the lncRNA PVT1 decreases miR-214 levels, thereby relieving inhibition of p53 and TFR1 expression, and resulting in ferroptosis and brain tissue injury [93]. Interestingly, PVT1 is a p53-induced lncRNA [94]. Therefore, the PVT1/miR-214/p53 axis may form a positive feedback regulatory loop that exacerbates ischemia/reperfusion-caused brain damage, which should be avoided clinically. Analogously, in acute myeloid leukemia, the circular RNA circKDM4C disrupts the hsa-let-7b-5p/p53 axis by sponging this miRNA [58]. The increased level of p53 by circKDM4C promotes ferroptosis and inhibits xenograft tumor growth.

The p53/ferroptosis pathway can also be regulated by other proteins (Fig. 4E). In colon carcinoma, upregulated N-acylsphingosine amidohydrolase (ASAH2) destabilizes p53 and thus protects the myeloid-derived suppressor cells (MDSCs) against p53-triggered ferroptosis [95]. These MDSCs accumulate in the microenvironment of colon carcinoma to repress the T cell immune response. In the same study, researchers developed an ASAH2 inhibitor NC06 to effectively suppress its ceramidase activity and activate p53-Hmox1 axis to induce ferroptosis in

MDSCs, leading to significant tumor suppression in a preclinical mouse model. In lung cancer, p53 transcriptionally represses the expression of the RNA binding protein ELAV like RNA binding protein 1 (ELAVL1) [38]. Overexpression of lymphoid-specific helicase reverses this inhibition and upregulates ELAVL1. The increased ELAVL1 level stabilizes LINC00336 to abolish miR-6852-mediated CBS repression, thus increasing cancer cell resistance against ferroptosis. Cytoglobin (CYGB) is a ROS regulator and tumor suppressor. In colon cancer cells, CYGB activates ACSL4 expression through the CYGB/p53/YAP1/ACSL4 pathway, which boosts ferroptosis and tumor suppression [59]. Inhibitor of apoptosis-stimulating protein of p53 (iASPP) suppresses p53-activated apoptosis through direct p53 binding. Intriguingly, iASPP inhibits ferroptosis by regulating the NRF2 pathway in intestinal ischemia/reperfusion-induced acute lung injury [96]. Whether iASPP can impede p53-modulated ferroptosis in this context remains poorly understood.

The levels of some intracellular small molecules, ROS, and iron can influence the activity of p53 to regulate ferroptosis (Fig. 4D). As mentioned above, the P47S variant of p53 confers greater cell resistance to ferroptosis by increasing the levels of CoA and GSH [27]. Intriguingly, CoA and GSH perform feedback inhibition of p53, thereby impairing its oligomerization and activity, possibly through the interference of several critical cysteine residues on p53 by CoA and GSH. The decreased activity of p53 further inhibits ferroptosis. In lung cancer cells, the ROS elicited by Erastin treatment activates p53 via ATM-mediated p53 S15 phosphorylation to facilitate both ferroptosis and apoptosis [97]. p53 regulates cellular iron metabolism and consequently affects ferroptosis initiation [17]. Interestingly, intracellular iron levels in turn modulate p53 activity. In a sarcopenia model, iron overload in muscle cells has been found to activate ferroptotic cell death via the p53/SLC7A11 axis, which may be a mechanism underlying sarcopenia [98]. Similarly, in a PD model, treating dopaminergic cells with ferric ammonium citrate has been found to induce p53-dependent ferroptosis, which may further elicit apoptosis [99]. Targeting the p53-ferroptosis pathway may provide a novel therapeutic route for PD. Ferritin is an important protein for iron storage in cells. By decreasing labile iron levels, ferritin can inhibit ferroptosis [17]. In a middle cerebral artery occlusion rat model, ferritin decrease has been found to cause ferroptosis and cerebral ischemia-induced hippocampal neuronal death via activating the p53/SLC7A11 pathway [100]. Therefore, the p53-iron levels must be maintained in a delicate balance to avoid ferroptosis-related tissue injuries and disorders.

p53-related ferroptosis can also be triggered by various components in body fluids, including cytokines, nutrients, and waste (Fig. 4D). After high glucose or interleukin-1β treatment, ferroptosis is induced in human umbilical vein endothelial cells (HUVECs) owing to the activation of p53/SLC7A11 pathway [101]. Consistently, in the aorta in db/db mice, the mRNA levels of p53 and SLC7A11 are elevated and diminished, respectively. Simultaneously, de-endothelialized areas are found in the aortic endothelium in these mice. These data link p53-mediated ferroptosis and diabetes-induced endothelial dysfunction. When exposed to high concentrations of calcium oxalate (CaOx), ferroptosis is observed in HK-2 renal tubular epithelial cells [102] and is associated with elevated levels of p53, ACSL4, transferrin, and TfR, all of which are positive regulators of ferroptosis. Moreover, in vivo data have demonstrated that ferroptosis in these cells facilitates renal crystal deposition and the development of urolithiasis.

p53-ferroptosis pathway can even be affected by the light (Fig. 4D). Blue light (BL) is everywhere in our lives. A recent study revealed the biphasic effects of BL irradiation on HUVECs [103]. When the fluence of BL irradiation is low, it promotes viability, migration, and angiogenesis of HUVECs. However, high fluence of BL (HFBL) reverses these effects by inducing ferroptosis and necroptosis. Interestingly, a major molecular effect in the cells treated with HFBL is the activation of p53 signaling pathway. It is worth exploring whether the HFBL-activated ferroptosis is dependent on the induction of p53 pathway. This result issues a warning about the health relevance of the widely used BL sources including blue light-emitting diode equipment, given the light pollution is rapidly increasing these years [104]. In addition, BL can also be used for disease therapy via various mechanisms [105]. It will be attractive to use BL to modulate the p53-ferroptosis pathway in related disorders.

Taken together, the p53-ferroptosis pathway is regulated by a vast range of factors, thus providing extensive therapeutic opportunities for treating different diseases by targeting this pathway.

TARGETING P53-MEDIATED FERROPTOSIS FOR DISEASE TREATMENT

Ferroptosis is caused by the dysregulation of various metabolic pathways. In fact, the disruption of metabolic homeostasis is a shared hallmark of multiple diseases [106]. Therefore, the association between ferroptosis and the initiation and development of diverse disorders, including tumor, organ injury (particularly ischemic injury), degenerative diseases and aging, and immunological dysfunction, is not surprising [10]. As a master regulator of ferroptosis pathways, p53 participates in fine-tuning all pathological functions of ferroptosis. Consequently, targeting p53 and p53-mediated ferroptosis has great potential for treating these diseases. Theoretically, targeting the p53-ferroptosis pathway has at least three advantages. (1) p53 can manipulate both canonical and non-canonical ferroptotic pathways through various mechanisms. Targeting p53 alone is more efficient than targeting a batch of different regulators in distinct ferroptotic pathways. (2) Similarly, p53 is a pleiotropic protein that regulates not only ferroptosis but also a host of other important biological activities. A specific disorder is rarely rooted in just one mechanism. In this light, targeting a single pathway seldom completely cures a disease. Targeting p53 can have multiple outcomes including influencing ferroptosis, which may synergistically combat the disease. In tumor therapy, this aspect is critical, given that p53 is a powerful tumor suppressor in different types of tumors. Although ferroptosis induction may be the fundamental mechanism through which p53 represses tumor development [17], the contributions of other effects of p53 in this process should not be overlooked. Targeting p53 can orchestrate all these effects to repress tumor growth. (3) Targeting factors in p53-ferroptosis pathway may be more feasible than targeting factors in other ferroptosis pathways. For example, GPX4 knockout or inhibition may result in cell lethality. However, depletion of iPLA2β does not have clear effects on cell viability. Hence, targeting iPLA2β to promote cell ferroptosis and tumor suppression would lead to fewer adverse effects in the normal cells. Regarding the question of how to specifically target p53, several pathways have been suggested, and many promising drugs are emerging [107, 108], which will not be covered in detail in this review. In addition, several other drugs or therapeutics designed to target other pathways have been found to be involved in the p53-ferroptosis pathway. In the following part of this section, we describe in vitro and in vivo studies targeting p53-ferroptosis to efficiently treat various diseases, particularly tumors (Fig. 4F–I and Table 2).

Numerous exciting results support the promise of targeting the p53-ferroptosis pathway to treat diverse tumor types (Fig. 4F). The poly(ADP-ribose) polymerase 1 (PARP) inhibitor olaparib is an efficient targeted therapy drug for breast cancer type 1/2 susceptibility protein (BRCA)-mutated ovarian cancer. However, it does not substantially benefit patients with wild-type BRCA.

Table 2. Important reagents identified in p53-mediated ferroptosis.

Category of disease	Name of disease	Name of drug	Promote (+) or inhibit (–) p53-ferroptosis pathway	Mechanism of action	Note	Reference
Tumor	Ovarian cancer	Olaparib	+	Activate p53 to suppress SLC7A11	Effective in ovarian cancer cells with wild-type BRCA1/2	[109]
	Ovarian cancer	Superparamagnetic iron oxides (SPIO)	+	Synergize with p53 to promote ferroptosis		[133]
	Breast adenocarcinoma	Nitroisoxazole-containing spiro [pyrrolidin-oxindole] derivative 3d	+	Inhibit MDM2–p53 interaction and reduce GPX4 level		[110]
	Non-small cell lung cancer	Levobupivacaine	+	Increase p53 expression		[111]
	Lung cancer and colon cancer	BEBT-908	+	Hyperacetylate and activate p53	Enhance the efficacy of immunotherapy	[112]
	Prostate cancer	Flubendazole	+	Activate p53 to suppress SLC7A11	Effective in castration-resistant prostate cancer	[113]
	Gastric cancer	Tanshinone IIA	+	Activate p53 to suppress SLC7A11		[114]
	Tongue squamous cell carcinoma	Quisinostat	+	Activate p53		[115]
	Melanoma	Gambogenic acid	+	Reverse the suppressive effect of TGF-β1 on p53/SLC7A11 pathway	Effective in melanoma under epithelial-to-mesenchymal transition (EMT)	[116]
	Melanoma	DHA and EPA	+	Activate p53	Reduce bortezomib resistance	[121]
	Osteosarcoma	Bavachin	+	Abolish STAT3-mediated inhibition of p53/SLC7A11/GPX4 signaling and increases the intracellular level of labile iron		[117]
	Glioma	Pseudolaric acid B	+	Activate p53 to suppress SLC7A11		[118]
	Glioma	Newcastle disease virus (NDV)	+	Activate p53/SLC7A11/GPX4 pathway and induce ferritinophagy		[119]
	Renal cell carcinoma	Artesunate	+	Activate p53	Overcome the resistance to sunitinib	[120]
	Hepatocellular carcinoma and colon cancer	Oleanane triterpenoid saponin derivative D13	+	Activate p53	Overcome the multi-drug resistance	[122]
	Colon carcinoma	7-chloro-2-(3-chloroanilino)pyrano [3,4-e][1,3]oxazine-4,5-dione (NC06)	+	Inhibit ASAH2 to stabilize p53	May boost T cell–based immunotherapy in colon cancer	[95]
	Fibrosarcoma	Metal–organic network (MON) encapsulated with p53 plasmid (MON-p53)	+	Synergize with p53 to promote ferroptosis		[134]
	Nasopharyngeal carcinoma	Disulfiram/copper (DSF/Cu)	+	Increase p53 expression to activate p53/SAT1/ALOX15 pathway		[136]
Organ injury	Early brain injury (EBI)	Pifithrin-α	–	Inhibit p53	Improve post-subarachnoid hemorrhage (SAH) caused EBI	[124]
	Intracerebral hemorrhage (ICH)	Isorhynchophylline (IRN)	–	Activate miR-122 to inhibit p53/SLC7A11 pathway		[125]
	Cardiotoxicity	Etoposide (ETP)	+	Activate p53	Contributes to ETP-caused cardiotoxicity	[126]

Table 2. continued

Category of disease	Name of disease	Name of drug	Promote (+) or inhibit (–) p53-ferroptosis pathway	Mechanism of action	Note	Reference
	Liver fibrosis	Artemether	+	Activate p53 to suppress SLC7A11	Alleviate liver fibrosis	[129]
	Acute kidney injury (AKI)	A-lipoic acid (LA)	–	Inhibit p53 and reduce cellular labile iron level	Reverse folic acid (FA)-induced AKI	[130]
Degenerative disease and ageing	Parkinson's disease	Clioquinol (CQ)	–	Inhibit p53		[127]
	Parkinson's disease	MPTP (MPP^+)	+	Activate p53	Promote PC12 cell senescence and may lead to Parkinson's disease	[128]
Malaria infection	*Plasmodium* liver stage infection	Nutlin-3	+	Activate p53/SLC7A11/GPX4 pathway	Decrease the *Plasmodium* liver stage infection	[132]
Other	Human umbilical vein endothelial cells (HUVECs)	Zinc oxide nanoparticles (ZnO NPs)	+	Activate p53		[135]
		High fluence of blue light (HFBL)	+	Activate p53		[103]

Recently, Hong et al. demonstrated that olaparib treatment induces ferroptosis in ovarian cancer cells by activating the p53/SLC7A11 pathway [109]. Combined use of olaparib and FIN significantly decreases the tumor burden in mice. This research has identified a novel pathway underlying the effect of olaparib that is based on p53-induced ferroptosis and is different from the classic mechanism of interfering with DNA damage repair. As discussed before, ASAH2 inhibitor NC06 was used to activate p53-Hmox1 axis to trigger ferroptosis in MDSCs in colon carcinoma [95]. In breast adenocarcinoma cells, Liu et al. successfully synthesized a dual inhibitor of MDM2 and GPX4, a nitroisoxazole-containing spiro[pyrrolidin-oxindole] derivative 3d, which effectively activates p53- and GPX4-mediated ferroptosis and tumor suppression [110]. In non-small cell lung cancer, the local anesthetic Levobupivacaine increases p53 expression, enhances ferroptosis, and inhibits tumor growth [111]. A dual PI3K/HDAC inhibitor BEBT-908 activates immunogenic ferroptosis in lung cancer and colon cancer cells partially by hyperacetylating and activating p53 [112]. Interestingly, the anti-malarial drug Flubendazole activates p53 in castration-resistant prostate cancer (CRPC), thus inducing cell cycle arrest and ferroptosis [113]. The mechanism underlying the ferroptosis here is based on the inhibition of SLC7A11 and GPX4 by p53. In addition, Flubendazole synergizes with 5-fluorouracil in CRPC treatment. Similarly, Tanshinone IIA, a Chinese herb-derived pharmacologically active component, amplifies the p53/SLC7A11 axis and induces ferroptosis in gastric cancer cells [114]. In tongue squamous cell carcinoma, the histone deacetylase inhibitor quisinostat activates multiple death types of tumor cell including apoptosis, pyroptosis, and ferroptosis [115]. Especially, quisinostat-induced ferroptosis relies on the upregulation of p53 and downregulation of GPX4. In transforming growth factor beta 1 (TGF-β1)-stimulated melanoma cells, TGF-β1 causes epithelial-to-mesenchymal transition and resistance to ferroptosis partially via inhibition of p53 [116]. Gambogenic acid reverses the suppressive effect of TGF-β1 on p53. Activated p53 then triggers ferroptosis by downregulating SLC7A11. In osteosarcoma cells, bavachin treatment kills cell by inducing ferroptosis via two different ways [117]. On the one hand, bavachin abolishes STAT3-mediated inhibition of p53/SLC7A11/GPX4 signaling; on the other hand, bavachin increases the intracellular level of labile iron—these two mechanisms synergically promote ferroptosis and tumor repression. Pseudolaric acid B not only represses SLC7A11 by activating p53 but also increases the level of NOX4 in glioma [118]. Both activities cooperatively enhance ferroptotic cell death and tumor inhibition. Oncolytic virus Newcastle disease virus (NDV) can also be used to treat glioma [119]. Kan et al. recently proved that NDV activates p53/SLC7A11/GPX4 pathway to initiate ferroptosis in human glioma cell line U251. Meanwhile, NDV also promotes ferritinophagy to increase the level of labile iron, which enhances p53-triggered ferroptosis and tumor suppression.

Targeting the p53-ferroptosis pathway can also be effective in some types of therapy-resistant (including drug-resistant and radioresistant) tumors (Fig. 4F). As discussed above, PARP inhibition activates ferroptosis in ovarian cancer cells with wild-type BRCA1/2 in a p53-dependent manner [109]. Combination treatment with olaparib and FINs can overcome the olaparib resistance of these cells. Flubendazole is effective in CRPC by evoking p53-mediated cell cycle arrest and ferroptosis [113]. In the renal cell carcinoma cell line KTCTL-26, artesunate treatment evokes ferroptosis, owing to the expression of p53, which suppresses the growth of the sunitinib–resistant counterpart of this cell [120]. Pretreating multiple melanoma cells with omega-3 PUFA DHA and EPA before bortezomib treatment effectively reduce bortezomib resistance and improve its efficacy [121]. The underlying mechanism for DHA/EPA here is not fully elucidated, but may relate to p53-activated ferroptosis. More strikingly, the oleanane triterpenoid saponin derivative D13 exhibits significant

proliferation-inhibitory effects in several multi-drug-resistant cancer cells by simultaneously activating p53-dependent ferroptosis and apoptosis [122]. Furthermore, Lei et al. demonstrated that p53-triggered ferroptosis is an important determinant of the radiosensitivity in tumors bearing wild-type p53 [123]. This effect partly results from the downregulation of SLC7A11 by p53. p53 knockout in these cells leads to enhanced resistance to radiotherapy.

In tumor cells, p53-mediated ferroptosis kills cells and suppresses tumor growth. However, if this pathway is aberrantly activated in normal cells, it may cause injury to host organs or result in degenerative diseases and aging (Fig. 4G, H). Post-subarachnoid hemorrhage (SAH) can lead to early brain injury (EBI), which is associated with SAH-induced ferroptosis. A recent study has demonstrated that using a p53 inhibitor pifithrin-α to block the p53-regualated ferroptosis after SAH effectively improves the EBI [124]. In another intracerebral hemorrhage (ICH) model, isorhynchophylline, a component isolated from a Chinese herb, has been found to inhibit p53 expression by activating miR-122 [125], thus resulting in the derepression of SLC7A11 and the suppression of ICH-induced ferroptosis, and protecting neurocytes. Etoposide (ETP) is a widely used chemotherapy drug in diverse tumor types. However, the cardiotoxicity caused by ETP is a major adverse effect that limits its use. ETP-induced apoptosis has long been known to cause cardiotoxicity. A study by Nemade et al. has determined that ETP treatment activates p53-modulated ferroptosis and thus greatly contributes to cardiotoxicity [126]. The ferroptosis inhibitor Liproxstatin-1 results in significant recovery of dysfunctional human pluripotent stem cell-derived cardiomyocytes. Increased neuronal cell death and senescence are major causes of PD. Shi et al. shown that clioquinol (CQ) had therapeutic benefits in a monkey model of PD by activating the AKT serine/threonine kinase 1 (AKT)/mechanistic target of rapamycin kinase (mTOR)/p53 signaling pathway [127]. AKT and mTOR effectively repress p53-mediated apoptosis and rescue motor and non-motor deficits. Moreover, ferroptosis is another factor of cell death in PD. CQ might also regulate p53-induced ferroptosis in PD. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and its active metabolite 1-methyl-4-phenylpyridinium (MPP^+) induce oxidative stress and cell death in PD. A quite recent study demonstrated that MPP^+-activated p53/SLC7A11/GPX4 axis is responsible for MPP^+-triggered senescence of PC12 cells via inducing ferroptosis [128]. As discussed above, $p53^{3KR}$ promotes the premature aging phenotype by stimulating ferroptosis in mice on the background of $XRCC4^{-/-}$ genomic instability [24]. Interestingly, aging is also a key factor for PD. Therefore, the development of drugs blocking p53-mediated ferroptosis may not only delay aging, but also benefit the PD patients. In some situations, p53 can protect organs against injury by promoting ferroptosis. In a CCl_4-induced liver fibrosis model, artemether treatment significantly attenuates HSC activation and fibrotic scar formation, partly by activating the p53/SLC7A11/ferroptosis pathway [129]. Folic acid (FA) can cause acute kidney injury (AKI) by disrupting cellular redox homeostasis and activating ferroptosis. Li et al. demonstrated that A-lipoic acid (LA) could effectively reverse FA-induced AKI through suppression of ferroptosis [130]. Mechanistically, LA not only reduces cellular labile iron level, but also inhibits p53 activation. The latter action ensures the expression of SLC7A11 and the biogenesis of GSH.

p53-mediated ferroptosis is also involved in the pathology of malaria [28, 131, 132] (Fig. 4I). In 2013, a study showed that hepatic p53 activation restricts *Plasmodium* liver stage infection [131]. Mechanistically, after *Plasmodium* infection, p53 promotes ferroptosis in infected cells, thus eliminating those cells together with the parasite via the p53/SLC7A11/GPX4 pathway [132]. Targeting this pathway with Nutlin-3 or Erastin efficiently decreases the *Plasmodium* liver stage burden. Intriguingly, the p53 single-nucleotide polymorphism P47S found in many people of African descent has a less protective effect on *Plasmodium* infection, owing to a defect in inducing ferroptosis [28]. However, $p53^{P47S}$ mice evoke a stronger response to the malarial toxin hemozoin via the elevated level of M2-polarized macrophages in these mice. Thus, the p53-ferroptosis pathway has evolved as an effective anti-parasite immunological barrier.

Metal-based drugs have been developed to induce ferroptosis for the treatment of various diseases. p53-mediated ferroptosis contributes to the effects of some of these drugs (Fig. 4F). Superparamagnetic iron oxides (SPIO), incubated with human serum, trigger ferroptosis in ovarian cancer cells [133]. Overexpression of p53 at the same time significantly enhances SPIO-mediated ferroptosis and ovarian cancer inhibition. A metal–organic network (MON) is suitable for inducing Fenton reactions. One group has designed a nanomaterial encapsulated MON with p53 plasmid (MON-p53) [134]. When delivered into tumor cells or tumor-bearing mice, MON-p53 efficiently promotes both ferroptosis and apoptosis, thus suppressing tumor growth. Metals other than iron can be used to trigger ferroptosis [135, 136]. Zinc oxide nanoparticles (ZnO NPs) can evoke ferroptosis by modulating both ROS and iron metabolism in cells [135]. Interestingly, ZnO NPs activate p53. Knockdown of p53 by siRNA greatly diminishes the ferroptosis-promoting effect of ZnO NPs, thus suggesting that p53 largely contributes to the ZnO NPs-induced ferroptosis. Disulfiram/copper (DSF/Cu) has tumor-suppressive effects in a broad spectrum of tumor types. Li et al. discovered that the mechanism underlying DSF/Cu's eradication of tumor cells is associated with the induction of ferroptosis, which is partially regulated by p53 [136]. In nasopharyngeal carcinoma cells, DSF/Cu treatment increases p53 levels. Elevated p53 transactivates SAT1, thus boosting ferroptosis via increasing ALOX15. Notably, DSF/Cu does not change the level of SLC7A11, thus indicating that the effect of p53 is dependent not on SLC7A11 inhibition but on the SAT1/ALOX15 axis.

When choosing drugs to target the p53-ferroptosis pathway, a caveat exists in that some classic p53 activators induce ferroptosis in a p53-independent manner. The multi-kinase inhibitor drug Sorafenib activates p53 and evokes ferroptosis [137, 138]. Werth et al. performed a quantitative screen for changes in intracellular phosphorylation after Sorafenib treatment in the hepatocellular carcinoma (HCC) cell line SKHep1 [139]. However, in the first 120 min after treatment, the levels of total p53 and p53 S392 phosphorylation (an activating modification for p53 function) both decreased. This finding is inconsistent with the ferroptosis-promoting role of Sorafenib and p53. There are at least two explanations for this result. One possibility is that the level of p53 is augmented later than 120 min. The other possibility is that Sorafenib activates ferroptosis in SKHep1 independently of p53. APR-246 is a drug used to restore the normal function of mutant p53. In acute myeloid leukemia, APR-246 activates ferroptosis regardless of the mutational status of p53 [140]. The underlying mechanism may be direct binding and depletion of GSH by APR-246, which is independent of p53 [141]. Similarly to the APR-246 case, another p53 activator, Quercetin, facilitates the induction of ferroptosis in various cancer cells by regulating iron metabolism [142]. Again, this effect is dependent not on p53 but on TFEB. Therefore, elaborate experiments should be designed and performed to determine the exact mechanisms of these ferroptosis-inducing drugs.

CONCLUDING REMARKS AND FURTHER PERSPECTIVES

After more than 40 years of research, the critical role of p53 as a tumor suppressor is indisputable. However, debates continue regarding which function is exactly used by p53 as its "ultimate weapon" in suppressing tumor growth. Answering this question is

not only important for understanding the mode of p53-mediated tumor suppression but also necessary for developing tumor therapeutics by targeting p53. Historically, the ability to induce cell cycle arrest, senescence, and apoptosis has been considered the major function of p53 to limit tumor development. This paradigm has been challenged by in vivo evidence [14–16]. These activities are now considered the core effects of the p53 response to DNA damage (or some other stresses), but may be dispensable for repression of tumor growth. The p53 field must search for the next answer to this critical question. The identification of a novel RCD modality, ferroptosis, and the demonstration that p53 has pivotal effects in regulating ferroptosis have shed new light on this issue.

Ferroptosis is rooted in the dysregulation of cellular metabolism, including iron, lipid, amino acid, and ROS metabolism. Notably, metabolic reprogramming is a fundamental hallmark of tumor initiation and development. All four core metabolic elements in ferroptosis are tightly linked to tumor biology. The multipotent role of p53 in metabolic modulation makes it an ideal regulator of ferroptosis [17]. $p53^{3KR}$ and $p53^{4KR}$ mouse studies in our laboratory have also provided evidence supporting the speculation that promoting ferroptosis may be a fundamental way in which p53 suppresses tumor growth and progression [15, 79]. In this review, we briefly introduced the classical model of ferroptosis. Then we focused on how p53 mediates both canonical and non-canonical ferroptosis pathways, and how these processes are regulated. Finally, we discussed the existing ways to target p53-modulated ferroptosis for the treatment of various diseases, particularly tumor.

To conclude this review, we raise several key issues to which greater attention must be paid in future research (Box 3). In summary, the p53 and ferroptosis fields are rapidly developing, and many core issues remain to be addressed. Emerging research results have increasingly advanced our knowledge of p53 and ferroptosis. For both scientific and clinical reasons, these fields warrant much more research to reap more fruitful achievements.

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YL wrote the manuscript and drew the figures. WG revised the manuscript and figures. Both authors approved the submitted version.

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Correspondence and requests for materials should be addressed to Wei Gu.

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