Trends in Cell Biology

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Review

Emerging Mechanisms and Disease Relevance of Ferroptosis

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Cell death is an essential feature of development in multicellular organisms, a critical driver of degenerative diseases, and can be harnessed for treating some cancers. Understanding the mechanisms governing cell death is critical for addressing its role in disease. Similarly, metabolism is essential for normal energy and biomolecule production, and goes awry in many diseases. Metabolism and cell death are tightly linked in the phenomenon of ferroptosis, a form of regulated cell death driven by peroxidation of phospholipids. Glutathione peroxidase 4 (GPX4) uses glutathione to protect cells from ferroptosis by eliminating phospholipid peroxides. Recent data have revealed glutathione/GPX4-independent axes for suppressing ferroptosis, and insight into the regulation of iron and mitochondria in ferroptosis. Ferroptosis has recently been implicated in multiple diseases, and functions as a tumor suppression mechanism. Ferroptosis induction is a promising approach in treating several conditions, including neoplastic diseases. Here, we summarize these recent advances.

Highlights

There are three parallel pathways for defending against cell death by ferroptosis.

Iron availability is controlled by cellular iron import, storage, and efflux mechanisms.

Several tumor suppressor genes normally prevent tumor development by activating ferroptosis in developing tumor cells.

As cancers evolve to a more aggressive state, they become increasingly sensitive to ferroptosis.

The immune system uses ferroptosis as a means of eliminating cancer cells.

Ferroptosis Is a Recently Discovered Form of Cell Death That Is Controlled by Numerous Metabolic Pathways

Cells are the fundamental organizing unit of biological systems. The mechanisms governing the division, growth, proliferation, and death of cells are central to understanding the logic underlying the functioning of life on Earth, the possibility of life elsewhere in the universe, and the processes by which disease develops. Ferroptosis is a recently described form of cell death involving iron-dependent damage to membrane lipids; numerous metabolic pathways involving iron, lipids, and amino acids control the sensitivity of cells to ferroptosis. Over the past few years, numerous advances in understanding ferroptosis have shed light on the mechanisms by which ferroptosis is triggered, how cells protect themselves from ferroptosis, and the disease contexts in which ferroptosis is relevant. In this review, we summarize these recent advances and highlight future areas of ferroptosis research relevant to understanding the normal functions of ferroptosis in living systems, how ferroptosis contributes to disease, and how controlling ferroptosis may be harnessed to create new therapies. In particular, we discuss new advances in understanding how iron availability is regulated during ferroptosis, how membrane lipid oxidation is controlled, and how ferroptosis contributes to normal tumor suppression and ischemic organ injury.

Brief History of Ferroptosis

Cell death is critical for the normal development of multicellular organisms, and is aberrantly activated or suppressed in a large number of diseases [1]. Cell death was historically considered to be unregulated, until the 1950s, when the concept of 'programmed cell death' emerged, which led in subsequent decades to the discovery of apoptosis as a form of programmed cell death, along with expanding insight into its mechanisms of operation. For a time, apoptosis was synonymous with programmed cell death; however, during the early 2000s, the concept of programmed necrosis was established, leading to the emergence of cell death modalities

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that are molecularly controlled and, therefore, regulated, regardless of whether they are developmentally programmed [2]. Hence, the term 'regulated cell death' was coined to refer to such modes of cell death, including necroptosis and pyroptosis [2].

In parallel with these advances in understanding the regulation of cell death writ large, in 2003, the discovery of a small molecule, named erastin, was reported that could selectively kill engineered tumor cells through a nonapoptotic mechanism [3]. Over the next 9 years, it emerged that this compound, and several additional compounds discovered in the same screening system, could activate an iron-dependent form of cell death that showed the morphology of necrosis, but was distinct mechanistically from apoptosis and several well-characterized forms of necrosis, including necroptosis and pyroptosis [4–6]. This form of cell death was termed ferroptosis, because of its iron dependence. The initial pathway for inducing ferroptosis, which was worked out in 2012–2014, was that some cells basally accumulate lipid peroxides, and that turning off the defense system that eliminates these lipid peroxides would cause their accumulation to lethal levels. For example, starving cells of cysteine through inhibition of the cystine-glutamate antiporter, known as system $x_{\overline{c}}$, depletes the antioxidant peptide glutathione from cells and results in failure of the glutathione-dependent peroxidase GPX4, resulting in the accumulation of lethal levels of peroxidized lipids [7]. Despite the powerful insights provided by these discoveries, several key questions remained, namely, which specific lipids undergo oxidation to drive ferroptosis, and what other mechanisms beyond GPX4 and glutathione might some cells use to prevent ferroptosis. Over the past several years, answers to these questions have emerged, yielding a more precise understanding of the pathways that execute and regulate ferroptosis; these advances in our understanding are described herein.

Ferroptosis Is Regulated by Numerous Pathways and Implicated in an Increasing Number of Diseases

Ferroptosis is a form of cell death first reported in 2012 [4], although many of the processes involved in ferroptosis had been observed in isolation decades earlier, but not integrated into a unified process [8]. The mechanisms governing ferroptosis that were elucidated in the first few years after it was discovered centered around cysteine and glutathione metabolism, and the ability of the phospholipid peroxidase GPX4 to prevent the accumulation of peroxidized lipids [9], which built on early work on the basic functions of these molecules. During the 1950s, Harry Eagle and his colleagues determined that cysteine was needed for the survival of many cell lines; cells deprived of cysteine died by a morphology that was different from that caused by deprivation of other amino acids, but similar to that caused by some viral infections [10,11]. During the 1970s, necrotic cell death in the liver, dependent on cysteine and involving glutathione depletion, was reported [12–15]. Also during the 1970s, Shiro Bannai and colleagues reported that cell death induced by a lack of glutathione and cysteine could be suppressed by a-tocopherol, an inhibitor of lipid peroxidation [16]. Then, in 1982, Ursini and colleagues isolated the enzyme that became known as GPX4, which could suppress iron-catalyzed lipid peroxidation in membranes [17] and, during the following decade, GPX4 was shown to protect against cell death associated with lipid peroxidation [18] and oxidative stress [19].

The biological and pathological contexts in which ferroptosis might operate were at first poorly defined. Over the past few years, numerous studies have revealed that the mechanisms by which ferroptosis can be induced and suppressed are more diverse than originally supposed, extending beyond the glutathione–GPX4 axis (Figure 1). In addition, several biological and pathological contexts in which ferroptosis operates have been clarified, including its role in tumor suppression and organ damage.

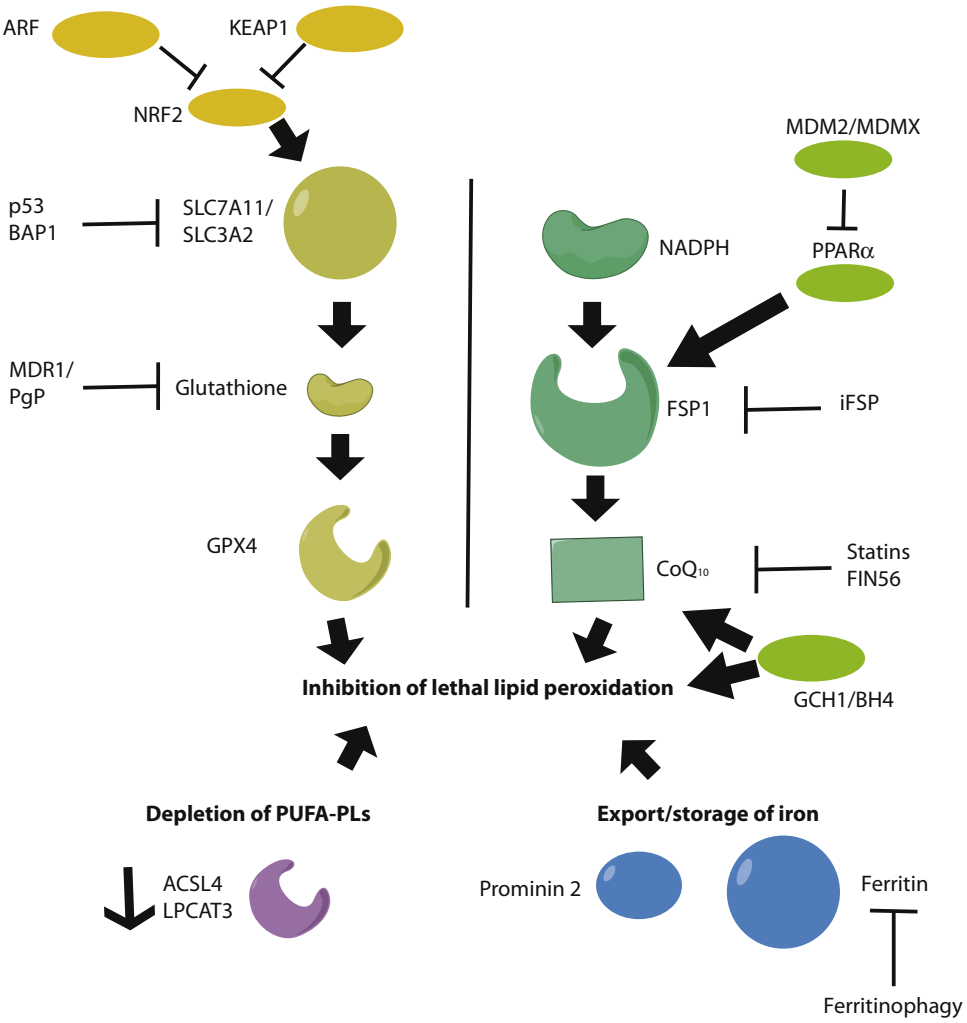

Figure 1. Key Regulators of Ferroptosis. Three distinct processes can drive resistance to ferroptosis. Depletion of polyunsaturated fatty acyl phospholipids (PUFA-PLs, purple, lower left) depletes the key substrates needed for lethal lipid peroxidation. Depletion of iron by storage in ferritin or export driven by prominin 2 blocks the iron-dependent peroxidation of PUFA-PLs (blue, lower right). Finally, three parallel pathways act to suppress lipid peroxidation by intercepting intermediates in the process: the glutathione pathway (left), the ubiquinone pathway [middle; NADPH–ferroptosis suppressor protein 1 (FSP1)–coenzyme (Co)Q_{10}], and the tetrahydrobiopterin [GTP cyclohydrolase 1 (GCH1)–tetrahydrobiopterin (BH4)] pathway (right). These are further regulated by upstream genes and proteins as indicated. Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; ARF, alternative reading-frame protein; LPCAT3, lysophosphatidylcholine acyltransferase 3; KEAP1, Kelch ECH-associated protein 1; MDR1, multidrug-resistant 1; NRF2, nuclear factor erythroid 2-related factor 2; PPAR, peroxisome proliferator-activated receptor; PgP, p-glycoprotein; SLC7A2/11, solute carrier family 7 member 2/11.

Regulation of Iron in Ferroptosis

Despite incorporating iron in the name, the role of and regulation of iron in ferroptosis only emerged recently. Initially, confusion surrounded as to whether the lipid peroxidation that drives ferroptosis was caused by the labile iron pool reacting with lipid peroxides to propagate these species in membranes, or whether iron-dependent enzymes might solely drive this peroxidation process. Studies from several laboratories revealed that iron-dependent lipoxygenases often initiate ferroptosis by causing the appearance of lipid peroxides, and labile iron (not bound to enzymes) propagates

these peroxides to drive overwhelming lipid peroxidation [20,21]. It remains possible that other iron-dependent enzymes contribute to lipid peroxidation in some circumstances; recently, cytochrome P450 oxidoreductase was implicated as a driver of lipid peroxidation during ferroptosis [22].

Ferritin is used to store Fe(III) in an inert form, where it cannot contribute to lipid peroxidation. Hence, the abundance of ferritin is a key factor governing sensitivity to ferroptosis: more ferritin results in more iron(III) storage and greater resistance to ferroptosis, as the labile iron pool becomes scarce. Conversely, depletion of ferritin results in the release of iron into the labile iron pool, resulting in greater sensitivity to ferroptosis. It has been demonstrated that ferritin-targeted autophagy, also known as ferritinophagy, results in the lysosomal degradation of ferritin and release of iron into the labile iron pool, thus causing increased sensitivity to ferroptosis [23,24]. A recent report revealed another iron-regulating process, in which prominin 2 drives ferroptosis resistance by promoting the formation of ferritin-containing multivesicular bodies and exosomes, which transport iron out of cells [25].

Lipid Peroxide Regulation

As noted earlier, the glutathione-dependent phospholipid peroxidase GPX4 was the first-discovered central inhibitor of ferroptosis [9]. GPX4 is a selenoprotein, implying that selenium availability impacts the sensitivity to ferroptosis. Indeed, delivery of selenium to cells or animals suppresses ferroptosis, including in a mouse model of intracerebral hemorrhage [26–28].

A genetic screen for regulators of ferroptosis sensitivity revealed the surprising finding that the multidrug resistance pump P-glycoprotein (PgP) normally pumps glutathione out of the cell, resulting in collateral sensitivity to ferroptosis in MDR1/PgP-expressing cells that are otherwise multidrug resistant [29]; this is consistent with an emerging picture in which ferroptosis sensitivity and traditional chemoresistance often co-occur.

In 2016, a new chemical inducer of ferroptosis, named FIN56, was reported [30]. FIN56 appeared to induce ferroptosis through a dual mechanism of depleting GPX4 protein and mevalonate pathway-derived coenzyme (Co)Q_{10}. CoQ_{10} is not only a critical part of the mitochondrial electron transport chain, but also functions outside of mitochondria to suppress lipid peroxidation by capturing radical intermediates in the process. Thus, depletion of CoQ_{10} sensitizes cells to ferroptosis. For example, statins, which inhibit mevalonate-derived CoQ_{10} by blocking the enzyme HMG CoA reductase, also sensitize cells to ferroptosis [30]. Two recent papers reported screens for genes that could suppress ferroptosis in the absence of GPX4, and discovered that AIFM2, now renamed ferroptosis suppressor protein 1 (FSP1), blocks lipid peroxidation and suppresses ferroptosis by regenerating reduced CoQ_{10}, independently of any need for GPX4 or glutathione [31,32]. This illuminated a new NADPH–FSP1–CoQ_{10} ferroptosis surveillance pathway that acts in parallel to glutathione–GPX4 to suppress ferroptosis. In addition to this pathway, a recent study identified another GPX4-independent ferroptosis-blocking pathway involving the gene GTP cyclohydrolase 1 (GCH1), which is the rate-limiting step in the production of the metabolite tetrahydrobiopterin (BH4) [33]. BH4 was found to suppress ferroptosis by aiding the formation of reduced CoQ_{10}, and by blocking the peroxidation of specific lipids. This provides another independent mechanism by which some cells prevent death through ferroptosis.

Ferroptosis as a Tumor Suppression Mechanism

While illuminating the basic mechanisms by which ferroptosis operates is of value for understanding fundamental cell biology, determining the natural functions of ferroptosis will aid in understanding how and why this form of cell death emerged during evolution. Accumulating evidence indicates that ferroptotic cell death leads to tumor growth suppression. Nevertheless, it remains to be further defined whether ferroptosis acts as a critical barrier to cancer

development. Inactivation of the p53 tumor suppression pathway is a pivotal event in the formation of most human cancers [34]. Although it is established that many activities of p53, including cell cycle arrest, senescence, and apoptosis, contribute to tumor suppression, accumulating evidence suggests that the combined loss of these activities of p53 is not sufficient to abrogate its tumor suppression activity [35]. Indeed, numerous studies indicate that p53-mediated metabolic regulation also promotes tumor suppression [36], but the mechanisms by which it does so remain unclear. Recent studies revealed that ferroptosis is one pathway connecting tumor suppression and metabolism. The tumor suppressor p53 has an important role in modulating ferroptotic responses through its transcriptional targets [37–39]. Indeed, $p53^{3KR}$ (3KR: K117R+K161R+K162R), an acetylation-defective mutant that fails to induce cell cycle arrest, senescence, and apoptosis, retains the ability to suppress tumor formation *in vivo* and to promote ferroptosis through metabolic regulation, including downregulation *of SLC7A11*, a key component of the cystine-glutamate antiporter [39]. Loss of an additional acetylation site at K98 (4KR: K98R+117R+K161R+K162R) abrogates the ferroptosis activity of p53, as well as its remaining tumor suppression function [40]. Thus, it is likely that ferroptosis has a key role in tumor suppression, particularly when cell cycle arrest, senescence, and apoptosis are not operative (Figure 2).

Interestingly, p53 is not the only tumor suppressor involved in regulating ferroptotic responses. It was reported that the BRCA1-associated protein 1 (BAP1) tumor suppressor is also able to promote ferroptosis by repressing *SLC7A11* [41]. Similar to p53, *BAP1* is a tumor suppressor that is frequently deleted or mutated in human cancers, including clear cell renal cell carcinoma, uveal melanoma, cholangiocarcinoma, and mesothelioma. Inactivation of BAP1 in cancer cells results in *SLC7A11* upregulation, ferroptosis resistance, and tumor development. Although the precise mechanism by which BAP1 induces *SLC7A11* repression needs further elucidation, it was proposed that BAP1, a H2A deubiquitinase, represses *SLC7A11* expression by regulating the levels of H2A ubiquitination (H2Aub) on the *SLC7A11* promoter (Figure 2) [41].

The above studies not only support the role of ferroptosis in tumor suppression under physiological settings, but also indicate the importance of SLC7A11 regulation in human cancers. SLC7A11 is a key component of a plasma membrane antiporter (the x_c^- system) that mediates Na^+-independent cellular uptake of extracellular cystine in exchange for intracellular glutamate [42–45]. *SLC7A11* overexpression is observed in many human cancers [46–48] and is induced under stress conditions, including oxidative stress. Nuclear factor erythroid 2-related factor 2 (NFR2) is one of the key regulators of the pathway to defend against oxidative stress [49]. It is normally kept at low levels by tumor suppressor Kelch ECH-associated protein 1 (KEAP1)-mediated ubiquitination. Under oxidative stress, NRF2 is stabilized and activated by dissociation from KEAP1 [49]. NRF2 is able to recognize the antioxidant response element (ARE) and activate several antioxidant genes, including *SLC7A11* [49].

Moreover, cancer cells rewire their cellular metabolism to meet the energetic and substrate demands of tumor development, generally accompanied by increased reactive oxygen species (ROS) production. Early studies showed that antioxidants may protect against cancer, because ROS and other free radicals may induce DNA damage and promote tumorigenesis [50,51]. However, large randomized clinical trials have produced inconsistent results, and suggest that antioxidants even increase cancer risk, although the precise mechanism remains unclear [52,53]. Notably, recent studies showed that the levels of NRF2 are induced upon oncogenic stress, and that several oncoproteins, including c-Myc, K-RAS, and B-Raf, promote tumor cell proliferation in part by stimulating NRF2-mediated expression of endogenous antioxidants and reducing ROS levels [54].

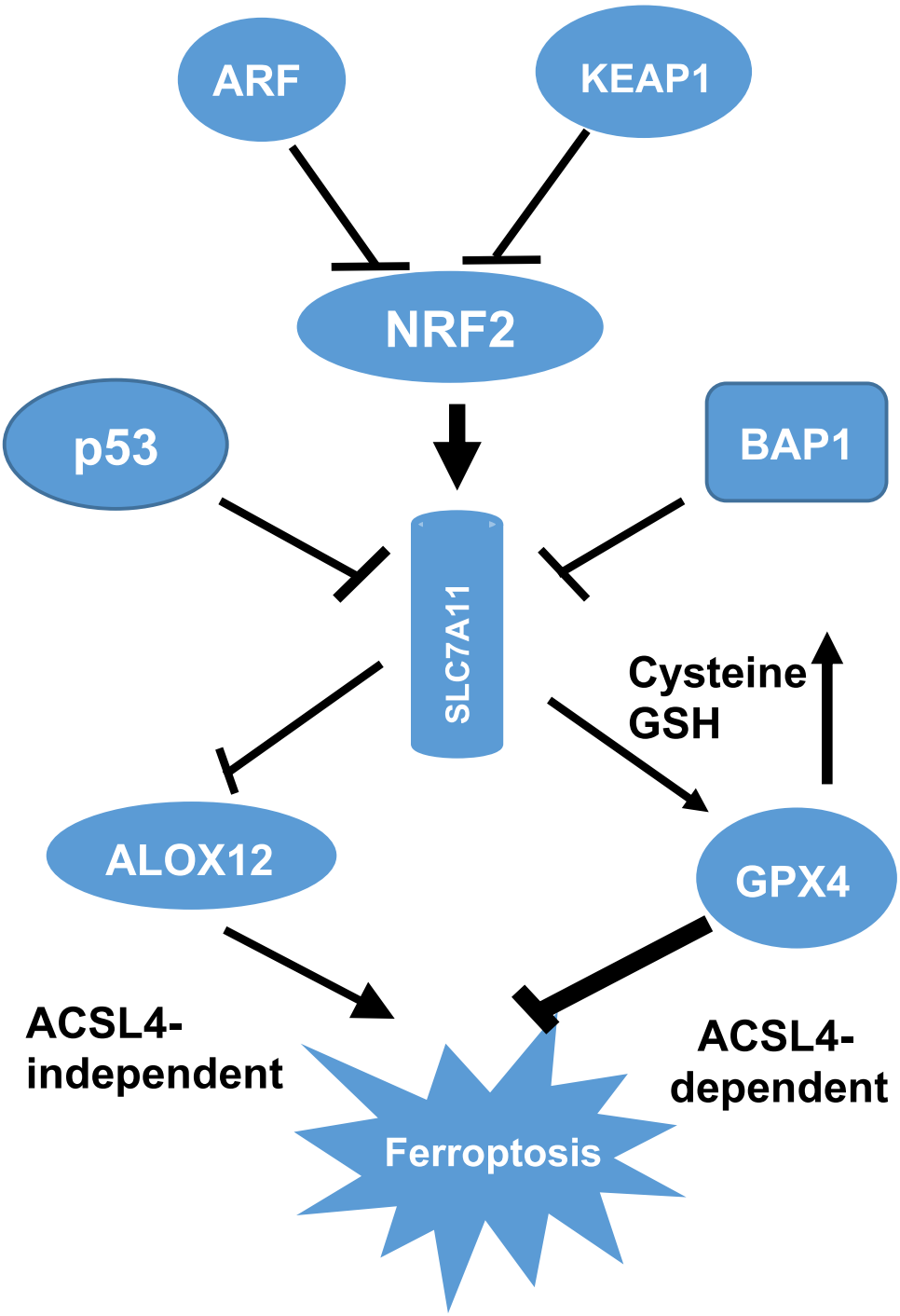

Figure 2. A Schematic Model for Tumor Suppressors Regulating Ferroptosis. The expression of solute carrier family 7 member 11 (SLC7A11) is a key mechanism by which numerous tumor-suppressor genes activate ferroptosis to prevent tumor formation. BRCA1-associated protein 1 (BAP1) and p53 repress SLC7A11, whereas suppressor Kelch ECH-associated protein 1 (KEAP1) and alternative reading-frame protein (ARF) prevent nuclear factor E2-related factor 2 (NRF2) from activating SLC7A11. Downstream of SLC7A11, arachidonate 12-lipoxygenase (ALOX12) and glutathione peroxidase 4 (GPX4) act in parallel pathways to regulate lipid peroxidation. Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; GSH, glutathione.

Moreover, genomic analyses of human cancers have also uncovered a high frequency of mutations associated with the NRF2–Keap1 axis, validating the *bona fide* oncogenic role of NRF2 *in vivo* [55]. Thus, it is important to know how the NRF2-mediated effect on ferroptosis is regulated in human cancers. ARF was initially identified as the product of an alternative reading frame within the Ink4a/ARF tumor suppressor locus [56]. ARF acts as a major regulator of p53 function in response to oncogenic stress by inhibiting the enzymatic activity of ubiquitin E3 ligases (i.e., Mdm2 and ARF-BP1) that target p53 for proteasomal degradation [57]. Surprisingly, through biochemical purification, ARF was found to be a key regulator of NRF2 [58]. ARF does not modulate NRF2 protein levels by interfering with KEAP1-mediated ubiquitination: instead, ARF inhibits CBP-dependent NRF2 acetylation and, therefore, significantly represses NRF2 transcriptional activity. As a consequence, ARF expression sensitizes cells to ferroptosis in a p53-independent manner, while ARF depletion induces NRF2 activation and promotes cancer cell survival in response to oxidative stress, suggesting that NRF2 is an important target of p53-independent tumor suppression by ARF. Together, these studies indicate that *SLCA711* expression is tightly regulated by both oncoproteins (e.g., NRF2) and tumor suppressors (e.g., p53, BAP1, and ARF) during stress responses (Figure 2), and that *SLC7A11* might be useful as a biomarker for ferroptosis *in vivo*.

Similar to p53 and BAP1, the tumor suppressor enzyme fumarase sensitizes cells to ferroptosis [59]. As an enzyme in the mitochondrial TCA cycle, fumarase catalyzes the conversion of fumarate to malate. Loss of function of fumarase can cause an increase in fumarate, which, through the inhibition of prolyl hydroxylases, enhances the stabilization of the proto-oncogenic hypoxia-inducible factor 1 alpha (HIF1α), which is involved in sensing and responding to oxygen concentrations. However, it has been shown that HIF1α and HIF2α are dispensable for the tumor-suppressive function of fumarase, leaving the mechanism of its tumor-suppressive function an enigma. More recently, it was demonstrated that the normal metabolic function of mitochondria, including the tricarboxylic acid (TCA) cycle in which fumarase is a key component, facilitates ferroptotic cell death in response to cysteine starvation (Figure 3) [59]. This finding provides a mechanistic explanation for the tumor-suppressive nature of fumarase (Figure 3). Intriguingly and similar to that of p53, the role of fumarase in ferroptosis appears to be context dependent; while loss of fumarase mitigates ferroptosis via interference with the TCA cycle, the resulting accumulation of fumarate has been reported to enhance succination and subsequent degradation of GPX4 [60], thus sensitizing cells to ferroptosis.

The precise and diverse mechanisms by which ferroptosis is induced under physiological settings remain to be elucidated. As noted earlier, it is established that ferroptosis is controlled by GPX4 [9]. The physiological significance of GPX4 in regulating ferroptosis is illustrated by the severe phenotypes associated with GPX4 mutant mice. For example, while *Gpx4*-null mice undergo embryonic lethality at 7.5 days post conception (dpc), conditional-*Gpx4*-mutant mice revealed that GPX4 loss not only induces ferroptosis, but also results in neurodegeneration [19], loss of antiviral immunity [61], infertility, and ischemia/reperfusion injury in kidney and liver [27,28,62,63].

In addition to GPX4-mediated neutralization of lipid peroxidation, the levels of cellular lipid peroxides can be enhanced enzymatically by lipoxygenases. Thus, lipoxygenases and GPX4 have opposite functions in ferroptosis: lipoxygenases cause the formation of lipid peroxides, while GPX4 eliminates them. Mice deficient for most of the lipoxygenase family members display a variety of physiological impairments, including defects in inflammation, ischemic cardioprotection, and airway epithelial injury in asthma. Both *Aloxe3*-deficient mice and *Alox12b*-deficient mice suffer postnatal death characterized by impaired barrier function of the skin, while *Alox15b*-deficient mice also display ichthyotic skin conditions similar to those of *Alox12b*- and *Aloxe3*-deficient

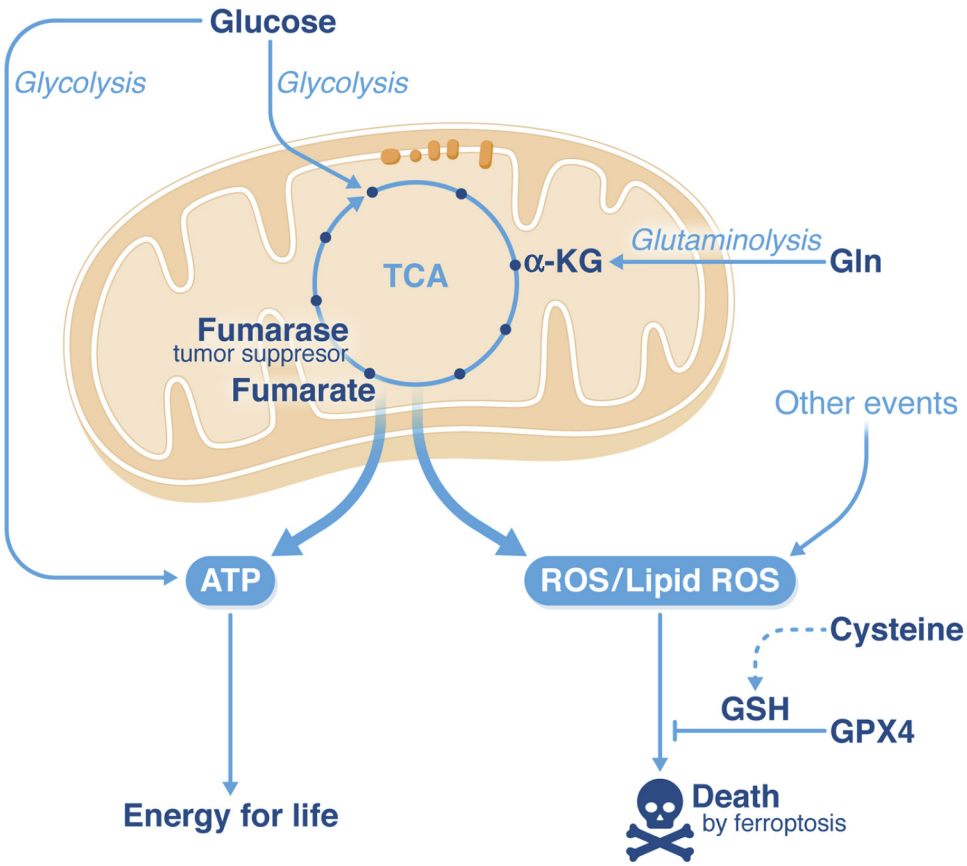

Figure 3. The Role of Mitochondria in Ferroptosis. Mitochondria, through the citric acid (TCA) cycle and electron transport chain activity, are the major source of cellular energy production. A side effect accompanying this oxidative process is the generation of reactive oxygen species (ROS), including ferroptosis-inducing lipid peroxides. Such a role for mitochondria in ferroptosis can explain the tumor-suppressive function of the TCA cycle enzyme fumarase. The blue arrows indicate the flow of reactions in metabolism, with glucose providing intermediates that enter the TCA cycle, glutamine (Gln) providing alpha-ketoglutarate (α-KG) via glutaminolysis, and then ATP and ROS flowing out of the TCA cycle. Abbreviations: GPX4, glutathione peroxidase 4; GSH, glutathione.

mice [63–67]. *Alox5*-deficient mice exhibit a suppressed response to inflammation, and reduced organ injury, including pancreases, lung, and liver, as well as amelioration in an Alzheimer's-disease-like phenotype [67–71]. Together, these studies reveal the physiological significance of both the activation and loss of ferroptotic responses *in vivo*.

Finally, the precise role of p53 in modulating ferroptotic responses is complex and requires further investigation. Initially, p53-dependent ferroptosis was thought to occur through prevention of cystine uptake and glutathione synthesis, which are required for GPX4 function, similar to the effect induced by erastin [39]. A recent report showed that ALOX12 is critical for p53-mediated ferroptosis. SLC7A11 interacts directly with ALOX12, but not with other ALOX family members, resulting in inhibition of ALOX12 activity [72]. Accordingly, p53 promotes ferroptosis through transcriptional repression of *SLC7A11*, which releases ALOX12 from SLC7A11-mediated inhibition (Figure 2). Notably, in contrast to GPX4 and the other lipoxygenase family members, deletion of *ALOX12* does not elicit major developmental defects in mice [72,73]. Indeed, *ALOX12*$^{+/-}$ and *ALOX12*$^{-/-}$ mice are fertile, healthy, and develop normally. Moreover, the *ALOX12* gene resides

on human chromosome 17p13.1 at a position close to the *TP53* gene. Loss of one *ALOX12* allele is sufficient to accelerate c-Myc-induced tumorigenesis in a haploinsufficient manner even when the classic functions of the p53 pathway (cell cycle arrest, apoptosis, and senescence) remain intact [72]. These studies provide genetic evidence that ferroptosis acts as a tumor-suppression mechanism independent of classic tumor-suppression mechanisms.

Recent studies showed that the ferroptotic responses induced by either erastin or GPX4 inhibitors are often dependent on acyl-CoA synthetase long-chain family member 4 (ACSL4) [74–76]. ACSL4 catalyzes the synthesis of long-chain polyunsaturated CoAs with a preference for arachidonic acid, thus facilitating their esterification into phospholipids. Consistent with these observations, further analyses showed that oxidized polyunsaturated fatty acid (PUFA)-containing phospholipids (PL-PUFA-OOH), but not free oxidized PUFAs (PUFA-OOH), act as the major executioners of ferroptosis [76]. It remains unclear why ACSL4 is specifically required for ferroptosis upon GPX4 inhibition, because other acyl-CoA synthetase long-chain family members are also able to induce esterification of PUFAs into phospholipids. Moreover, ferroptosis can occur in an ACSL4-independent manner, since ACSL4 is dispensable for the ferroptosis induced by the p53–ALOX12 axis [72]. Future investigations are required to examine whether other ACSL family members are involved in the esterification of phospholipids targeted by ALOX12.

Ferroptosis Modulation as a Therapeutic Avenue

Although a definitive physiological function for ferroptosis has yet to be unambiguously demonstrated, the role of ferroptosis in human diseases has been established. A wealth of studies suggests that pharmacological modulation of this unique cell death modality, by either inhibiting it or stimulating it, may yield significant clinical benefit for certain diseases.

Ischemic Organ Injuries and Degenerative Diseases

Ischemia is a major cause of a variety of devastating diseases, including ischemic heart disease, stroke, kidney failure, and liver damage. Notably, ischemic heart disease, for which effective therapies are lacking, results in more mortality annually than any other disease worldwide [77].

Pathologically, all ischemic organ injuries (IOI) share a similar symptom: massive cell death in affected organs. However, therapies that function by preventing IOI-associated cell death have not yet been developed, mainly due to the elusive mechanisms underlying ischemia-induced cell death. Over the past several years, mounting evidence has demonstrated that ferroptosis is a major contributor to IOI-associated cell death, and inhibition of ferroptosis significantly mitigated IOI in a cohort of experimental models. For example, in an *ex vivo* mouse heart model mimicking ischemia/reperfusion (IR), pharmacological inhibition of ferroptosis by an iron chelator and a glutaminase-2 inhibitor both reduced heart damage significantly, establishing the crucial role of ferroptosis in ischemic heart disease [38]. This conclusion was confirmed by a more recent *in vivo* study [78]. Conversely, induction of ferroptosis by conditional deletion of *GPX4* in mouse kidney caused kidney failure with pathological features similar to those seen in patients [62,79]. Taken together, these pharmacological and genetic studies indicate that ferroptosis inhibition is a promising approach for the treatment of IOI. Studies have shown that another type of regulated necrosis, necroptosis, may also contribute to IOI-associated cell death. However, many of these studies used a pharmacological inhibitor of necroptosis, necrostatin-1, which was also found to be able to inhibit ferroptosis [62].

Ferroptosis is also implicated in other forms of organ injury and degenerative disease. Recent studies suggest a role for lung epithelial cell ferroptosis in cigarette smoking-associated chronic obstructive pulmonary disease [80,81]. Additionally, studies using lipid peroxide-trapping agents and iron chelators support a role for ferroptosis in neurodegeneration [82]. Ferroptosis may also contribute to

glutamate excitotoxicity of neurons: by inhibiting the activity of system x_c^- cystine/glutamate antiporter, high doses of glutamate can prevent cellular cystine import, thus leading to the induction of ferroptosis [4].

Therapeutic Potential of Ferroptosis Induction in Cancer

A prominent role for ferroptosis in cancer development and treatment is emerging. We discussed earlier the potential physiological function of ferroptosis in tumor suppression and how cancer cells may bypass this tumor suppressive mechanism by genetic mutations. Strikingly, it has been demonstrated that numerous types of therapy-resistant cancer cell, especially those with mesenchymal and dedifferentiated characteristics, are more susceptible to ferroptosis [83–85]. These findings suggest that induction of ferroptosis is a promising cancer therapeutic approach, especially for the treatment of mesenchymal and metastatic cancers, which are often resistant to all available therapies.

A recent study revealed a novel, noncell autonomous mechanism for the regulation of ferroptosis that provides mechanistic insights into the basis for the enhanced sensitivity of drug-resistant cancer cells to ferroptosis [86]. In epithelial cells, E-cadherin-mediated intercellular interactions, signaling through the intracellular Merlin–NF2–Hippo pathway, inhibit the transcription co-activator YAP and its ferroptosis-potentiating activity (the study showed that two ferroptosis-promoting factors, ACSL4 [74] and transferrin receptor [38], are transcriptional targets of the YAP–TEAD4 complex). In epithelial cancer cells, decreased E-cadherin or NF2 expression, reduced Hippo pathway activity, and enhanced YAP activation can promote epithelial–mesenchymal transition (EMT) and metastasis [87,88]. Therefore, this finding explains why EMT/metastasis-prone cancer cells are highly susceptible to ferroptosis [83]. Furthermore, since the E-cadherin, NF2, and Hippo signaling components *Lats1* and *Lats2* are tumor suppressors frequently mutated in cancers that promote cancer progression, these malignant mutations might be Achilles' heels and could be used as biomarkers to predict cancer cell responsiveness to ferroptosis. In xenograft mouse models for mesothelioma (loss of function of NF2 mutation occurs in >35% of patients with mesothelioma), although loss of *NF2* function increases mesothelioma tumor growth and metastasis, it renders tumor cells more sensitive to GPX4 inactivation [86]. Consistent with this study, it was reported in a separate study that TAZ, a homolog of YAP often upregulated in renal cancer, is also able to sensitize renal cancer to ferroptosis induction [89].

Regulation of ferroptosis by a noncell autonomous mechanism also has intriguing implications beyond cancer biology. Ferroptosis, similar to other programmed cell death mechanisms, uses cell-intrinsic machinery for execution. Remarkably, in the context of ferroptotic cell death, neighboring cells can have a significant impact on decision making, via the E-cadherin–NF2–Hippo–YAP signaling axis. Such intercellular communication appears to be mutually beneficial, because it increases the resistance to ferroptosis of all involved cells. The consequence of this intercellular communication is in stark contrast to that of death receptor-mediated apoptosis [90,91], in which one cell (bearing the death receptor ligand) induces apoptotic death of the other (bearing the death receptor). Considering that multicellular organisms are under frequent insult from oxidative stress, this intercellular antiferroptotic mechanism might be another layer of crucial defense that cells use to protect themselves from ferroptosis, a terminal and irreversible consequence of oxidative stress.

GPX4 and the system x_c^- cystine-glutamate antiporter are two of the validated targets for inducing ferroptosis. While genetic deletion of *GPX4* causes lethality in mice [92], indicating the potential toxicity of its inhibition, there may still be a therapeutic window, as suggested by studies revealing differential sensitivity of cancer cells to GPX4 inhibition [9,83,84,86].

Inhibition of the system x_c^- cystine-glutamate antiporter will most likely be more tolerable, because mice with genetic deletion of *SLC7A11*, which encodes an essential subunit of the antiporter, are viable without obvious developmental defects [93,94]. Indeed, tumor xenograft experiments in mice using an erastin derivative with better pharmacological properties, imidazole ketone erastin (IKE), showed a clear therapeutic benefit without causing significant weight loss of mice, which is a general sign of toxicity [86,95]; this suggests that IKE will be well tolerated as a therapeutic agent. In addition, the newly discovered ferroptosis-inhibitory protein FSP1 provides another potential therapeutic target, considering that this protein is expressed in many cancer cells [31,32].

A combination of ferroptosis induction and other therapeutic approaches is also promising. It was reported recently that anti-PDL1 immune checkpoint blockade can elicit a cancer cell ferroptotic response through the downregulation of *SLC7A11* expression in cancer cells, which is a consequence of $CD8^+$ T cell-secreted IFNγ. These T cells are key components of the natural mechanism by which the immune system eliminates developing cancers. As such, a combination of anti-PDL1 treatment with ferroptosis induction was shown to have a synergistic anticancer effect in mouse models [96–98]. Ferroptosis has also been suggested to be partially responsible for the anticancer effect of radiation therapy [97,99,100]. All these recent advances underscore the cancer therapeutic potential of ferroptosis induction as a monotherapy and as a component of combination therapies.

Concluding Remarks and Future Perspectives

The rapid expansion of our understanding of ferroptosis is due to the growing number of laboratories that are exploring the mechanisms and functions of this form of cell death. Given the recent advances, we know that there are at least three major pathways that control sensitivity of cells to ferroptosis: the glutathione–GPX4, NADPH–FSP1–CoQ_{10}, and GCH1–BH4 pathways. Moreover, it appears clear that ferroptosis contributes to both degenerative disease pathology and tumor suppression, and that inhibiting and inducing ferroptosis have the potential to be therapeutic in disease settings. We now know that cells have evolved a complex set of pathways for controlling when and how ferroptosis is activated, and have harnessed this mode of cell death to prevent tumorigenesis. Numerous targets have been identified for inducing and inhibiting ferroptosis, but which of these provide the best therapeutic index, are translatable to animal models and patients, and are chemically tractable, is not clear.

Future research may shed light on other natural functions of ferroptosis, such as its role in preventing infections or in the normal development of tissues and organs. In addition, ferroptosis is more conserved throughout the diversity of life on earth than any other form of cell death and, thus, it will be interesting to compare how ferroptosis is regulated and used in diverse species.

Finally, it is still unknown exactly why and how lipid peroxidation leads to the death of cells in ferroptosis, and this is a key area of future research (see Outstanding Questions). We know that accumulation of oxidized phospholipids is a death signal, but we do not know how this causes cell death and whether it is possible to intervene in this process after these species have accumulated.

Key remaining questions for the field include: (i) what are the best scaffolds and mechanisms for therapeutically modulating ferroptosis; (ii) what are the mechanisms by which ferroptosis is executed downstream of phospholipid peroxidation; and (iii) has nature co-opted ferroptosis for normal physiological functions in any species? Given that new discoveries continue to emerge even regarding the mechanisms and function of apoptosis, the first-discovered form of regulated cell death that has been extensively studied over the past 50 years, there appears to be a vast untapped terrain of ferroptosis biology awaiting study.

Outstanding Questions

What are the best mechanisms for therapeutically modulating ferroptosis?

What are the mechanism by which ferroptosis is executed downstream of phospholipid peroxidation?

Has nature co-opted ferroptosis for normal physiological functions in other species?

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References

1. Fuchs, Y. and Steller, H. (2011) Programmed cell death in animal development and disease. *Cell* 147, 742–758
2. Galluzzi, L. *et al.* (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25, 486–541
3. Dolma, S. *et al.* (2003) Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell* 3, 285–296
4. Dixon, S.J. *et al.* (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060–1072
5. 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
6. Yagoda, N. *et al.* (2007) RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature* 447, 864–868
7. Dixon, S.J. *et al.* (2014) Pharmacological inhibition of cystine–glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *eLife* 3, e02523
8. Hirschhorn, T. and Stockwell, B.R. (2019) The development of the concept of ferroptosis. *Free Radic. Biol. Med.* 133, 130–143
9. Yang, W.S. *et al.* (2014) Regulation of ferroptotic cancer cell death by Gpx4. *Cell* 156, 317–331
10. Eagle, H. (1955) Nutrition needs of mammalian cells in tissue culture. *Science* 122, 501–514
11. Eagle, H. (1955) The specific amino acid requirements of a human carcinoma cell (Stain HeLa) in tissue culture. *J. Exp. Med.* 102, 37–48
12. Potter, W.Z. *et al.* (1973) Acetaminophen-induced hepatic necrosis. 3. Cytochrome P-450-mediated covalent binding *in vitro*. *J. Pharmacol. Exp. Ther.* 187, 203–210
13. Mitchell, J.R. *et al.* (1973) Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.* 187, 211–217
14. Mitchell, J.R. *et al.* (1973) Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Ther.* 187, 185–194
15. Jollow, D.J. *et al.* (1973) Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J. Pharmacol. Exp. Ther.* 187, 195–202
16. Bannai, S. *et al.* (1977) Effect of antioxidants on cultured human diploid fibroblasts exposed to cystine-free medium. *Biochem. Biophys. Res. Commun.* 74, 1582–1588
17. Ursini, F. *et al.* (1982) Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. *Biochim. Biophys. Acta* 710, 197–211
18. Geiger, P.G. *et al.* (1991) Lethal damage to murine L1210 cells by exogenous lipid hydroperoxides: protective role of glutathione-dependent selenoperoxidases. *Arch. Biochem. Biophys.* 288, 671–680
19. Seiler, A. *et al.* (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* 8, 237–248
20. Shah, R. *et al.* (2018) Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Cent. Sci.* 4, 387–396
21. Wenzel, S.E. *et al.* (2017) PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell* 171, 628–641
22. Zou, Y. *et al.* (2020) Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. *Nat. Chem. Biol.* 16, 302–309
23. Hou, W. *et al.* (2016) Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12, 1425–1428
24. Gao, M. *et al.* (2016) Ferroptosis is an autophagic cell death process. *Cell Res.* 26, 1021–1032
25. Brown, C.W. *et al.* (2019) Prominin2 drives ferroptosis resistance by stimulating iron export. *Dev. Cell* 51, 575–586
26. Alim, I. *et al.* (2019) Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell* 177, 1262–1279
27. Ingold, I. *et al.* (2018) Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* 172, 409–422
28. Friedmann Angeli, J.P. and Conrad, M. (2018) Selenium and GPX4, a vital symbiosis. *Free Radic. Biol. Med.* 127, 153–159
29. Cao, J.Y. *et al.* (2019) A genome-wide haploid genetic screen identifies regulators of glutathione abundance and ferroptosis sensitivity. *Cell Rep.* 26, 1544–1556
30. Shimada, K. *et al.* (2016) Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat. Chem. Biol.* 12, 497–503
31. Doll, S. *et al.* (2019) FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 575, 693–698
32. Bersuker, K. *et al.* (2019) The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 575, 688–692
33. Kraft, V.A.N. *et al.* (2020) GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Cent. Sci.* 6, 41–53
34. Kastenhuber, E.R. and Lowe, S.W. (2017) Putting p53 in context. *Cell* 170, 1062–1078
35. Kaiser, A.M. and Attardi, L.D. (2018) Deconstructing networks of p53-mediated tumor suppression *in vivo*. *Cell Death Differ.* 25, 93–103
36. Kung, C.P. and Murphy, M.E. (2016) The role of the p53 tumor suppressor in metabolism and diabetes. *J. Endocrinol.* 231, R61–R75
37. Jennis, M. *et al.* (2016) An African-specific polymorphism in the TP53 gene impairs p53 tumor suppressor function in a mouse model. *Genes Dev.* 30, 918–930
38. Gao, M. *et al.* (2015) Glutaminolysis and transferrin regulate ferroptosis. *Mol. Cell* 59, 298–308
39. Jiang, L. *et al.* (2015) Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520, 57–62
40. Wang, S.J. *et al.* (2016) Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. *Cell Rep.* 17, 366–373
41. Zhang, Y. *et al.* (2018) BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat. Cell Biol.* 20, 1181–1192
42. Lo, M. *et al.* (2008) The xc- cystine/glutamate antiporter: a mediator of pancreatic cancer growth with a role in drug resistance. *Br. J. Cancer* 99, 464–472
43. Conrad, M. and Sato, H. (2012) The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (–): cystine supplier and beyond. *Amino Acids* 42, 231–246
44. Ishimoto, T. *et al.* (2011) CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. *Cancer Cell* 19, 387–400
45. Sato, H. *et al.* (2000) Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc. *Antioxid. Redox Signal.* 2, 665–671
46. Huang, Y. *et al.* (2005) Cystine-glutamate transporter SLC7A11 in cancer chemosensitivity and chemoresistance. *Cancer Res.* 65, 7446–7454
47. Liu, X.X. *et al.* (2011) MicroRNA-26b is underexpressed in human breast cancer and induces cell apoptosis by targeting SLC7A11. *FEBS Lett.* 585, 1363–1367

48. Guo, W. *et al.* (2011) Disruption of xCT inhibits cell growth via the ROS/autophagy pathway in hepatocellular carcinoma. *Cancer Lett.* 312, 55–61
49. Jaramillo, M.C. and Zhang, D.D. (2013) The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev.* 27, 2179–2191
50. Chio, I.I.C. and Tuveson, D.A. (2017) ROS in cancer: the burning question. *Trends Mol. Med.* 23, 411–429
51. Willcox, J.K. *et al.* (2004) Antioxidants and prevention of chronic disease. *Crit. Rev. Food Sci. Nutr.* 44, 275–295
52. Alpha-Tocopherol, B.C.C.P.S.G (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.* 330, 1029–1035
53. Klein, E.A. *et al.* (2011) Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 306, 1549–1556
54. DeNicola, G.M. *et al.* (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475, 106–109
55. Hayes, J.D. and McMahon, M. (2009) NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. *Trends Biochem. Sci.* 34, 176–188
56. Sherr, C.J. (2006) Divorcing ARF and p53: an unsettled case. *Nat. Rev. Cancer* 6, 663–673
57. Kruse, J.P. and Gu, W. (2009) Modes of p53 regulation. *Cell* 137, 609–622
58. Chen, D. *et al.* (2017) NRF2 is a major target of ARF in p53-independent tumor suppression. *Mol. Cell* 68, 224–232
59. Gao, M. *et al.* (2019) Role of mitochondria in ferroptosis. *Mol. Cell* 73, 354–363
60. Kerins, M.J. *et al.* (2018) Fumarate hydratase inactivation in hereditary leiomyomatosis and renal cell cancer is synthetic lethal with ferroptosis induction. *Cancer Sci.* 109, 2757–2766
61. Matsushita, M. *et al.* (2015) T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. *J. Exp. Med.* 212, 555–568
62. Friedmann Angeli, J.P. *et al.* (2014) Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat. Cell Biol.* 16, 1180–1191
63. Imai, H. *et al.* (2017) Lipid peroxidation-dependent cell death regulated by GPx4 and ferroptosis. *Curr. Top. Microbiol. Immunol.* 403, 143–170
64. Moran, J.L. *et al.* (2007) A mouse mutation in the 12R-lipoxygenase, Alox12b, disrupts formation of the epidermal permeability barrier. *J Invest Dermatol* 127, 1893–1897
65. Yu, Z. *et al.* (2005) Mutations associated with a congenital form of ichthyosis (NCIE) inactivate the epidermal lipoxygenases 12R-LOX and eLOX3. *Biochim. Biophys. Acta* 1686, 238–247
66. Cuzzocrea, S. *et al.* (2003) 5-lipoxygenase knockout mice exhibit a resistance to acute pancreatitis induced by cerulein. *Immunology* 110, 120–130
67. Joshi, Y.B. and Pratico, D. (2011) Knockout of 5-lipoxygenase results in age-dependent anxiety–like behavior in female mice. *PLoS ONE* 6, e29448
68. Magnusson, L.U. *et al.* (2012) Arachidonate 15-lipoxygenase type B knockdown leads to reduced lipid accumulation and inflammation in atherosclerosis. *PLoS ONE* 7, e43142
69. Kinder, M. *et al.* (2010) Interferon regulatory factor-8-driven myeloid differentiation is regulated by 12/15-lipoxygenase-mediated redox signaling. *Exp. Hematol.* 38, 1036–1046
70. Reddy, M.A. *et al.* (2003) Reduced growth factor responses in vascular smooth muscle cells derived from 12/15-lipoxygenase-deficient mice. *Hypertension* 41, 1294–1300
71. Krieg, P. *et al.* (2013) Aloxe3 knockout mice reveal a function of epidermal lipoxygenase-3 as hepoxilin synthase and its pivotal role in barrier formation. *J Invest Dermatol* 133, 172–180
72. Chu, B. *et al.* (2019) ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. *Nat. Cell Biol.* 21, 579–591
73. Johnson, E.N. *et al.* (1998) Increased platelet sensitivity to ADP in mice lacking platelet-type 12-lipoxygenase. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3100–3105
74. Doll, S. *et al.* (2017) ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13, 91–98
75. Dixon, S.J. *et al.* (2015) Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem. Biol.* 10, 1604–1609
76. Kagan, V.E. *et al.* (2017) Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* 13, 81–90
77. Writing Group Members *et al.* (2016) Heart disease and stroke statistics-2016 update: a report from the American Heart Association. *Circulation* 133, e38–e360
78. Fang, X. *et al.* (2019) Ferroptosis as a target for protection against cardiomyopathy. *Proc. Natl. Acad. Sci. U. S. A.* 116, 2672–2680
79. Linkermann, A. *et al.* (2014) Synchronized renal tubular cell death involves ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 111, 16836–16841
80. Yoshida, M. *et al.* (2019) Involvement of cigarette smoke-induced epithelial cell ferroptosis in COPD pathogenesis. *Nat. Commun.* 10, 3145
81. Park, E.J. *et al.* (2019) Whole cigarette smoke condensates induce ferroptosis in human bronchial epithelial cells. *Toxicol. Lett.* 303, 55–66
82. Devos, D. *et al.* (2014) Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid. Redox Signal.* 21, 195–210
83. Viswanathan, V.S. *et al.* (2017) Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* 547, 453–457
84. Hangauer, M.J. *et al.* (2017) Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 551, 247–250
85. Tsoi, J. *et al.* (2018) Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. *Cancer Cell* 33, 890–904
86. Wu, J. *et al.* (2019) Intercellular interaction dictates cancer cell ferroptosis via NF2-YAP signalling. *Nature* 572, 402–406
87. Johnson, R. and Halder, G. (2014) The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment. *Nat. Rev. Drug Discov.* 13, 63–79
88. Overholtzer, M. *et al.* (2006) Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. *Proc. Natl. Acad. Sci. U. S. A.* 103, 12405–12410
89. Yang, W.H. *et al.* (2019) The Hippo pathway effector TAZ regulates ferroptosis in renal cell carcinoma. *Cell Rep.* 28, 2501–2508
90. Ashkenazi, A. and Dixit, V.M. (1998) Death receptors: signaling and modulation. *Science* 281, 1305–1308
91. Jiang, X.J. and Wang, X.D. (2004) Cytochrome C-mediated apoptosis. *Annu. Rev. Biochem.* 73, 87–106
92. Yant, L.J. *et al.* (2003) The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic. Biol. Med.* 34, 496–502
93. McCullagh, E.A. and Featherstone, D.E. (2014) Behavioral characterization of system xc- mutant mice. *Behav. Brain Res.* 265, 1–11
94. Sato, H. *et al.* (2005) Redox imbalance in cystine/glutamate transporter-deficient mice. *J. Biol. Chem.* 280, 37423–37429
95. Zhang, Y. *et al.* (2019) Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. *Cell Chem. Biol.* 26, 623–633
96. Wang, W. *et al.* (2019) CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature* 569, 270–274
97. Lang, X. *et al.* (2019) Radiotherapy and immunotherapy promote tumoral lipid oxidation and ferroptosis via synergistic repression of SLC7A11. *Cancer Discov.* 9, 1673–1685
98. Stockwell, B.R. and Jiang, X. (2019) A physiological function for ferroptosis in tumor suppression by the immune system. *Cell Metab.* 30, 14–15
99. Ye, L.F. *et al.* (2020) Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers. *ACS Chem. Biol.* 15 (2), 469–484
100. Lei, G. *et al.* (2020) The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression. *Cell Res.* 30 (2), 146–162