ANNUAL REVIEWS

Annual Review of Cancer Biology

The Hallmarks of Ferroptosis

Scott J. Dixon[1] and Brent R. Stockwell[2]

[1]Department of Biology, Stanford University, Stanford, California 94305, USA; email: sjdixon@stanford.edu

[2]Department of Biological Sciences and Department of Chemistry, Columbia University, New York, NY 10027, USA; email: bstockwell@columbia.edu

Annu. Rev. Cancer Biol. 2019. 3:35–54

First published as a Review in Advance on October 24, 2018

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-030518-055844

Keywords

glutathione, p53, cell death, chemotherapy, peroxidation, iron

Abstract

Ferroptosis is a nonapoptotic, iron-dependent form of cell death that can be activated in cancer cells by natural stimuli and synthetic agents. Three essential hallmarks define ferroptosis, namely: 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. Several processes including RAS/MAPK signaling, amino acid and iron metabolism, ferritinophagy, epithelial-to-mesenchymal transition, cell adhesion, and mevalonate and phospholipid biosynthesis can modulate susceptibility to ferroptosis. Ferroptosis sensitivity is also governed by p53 and KEAP1/NRF2 activity, linking ferroptosis to the function of key tumor suppressor pathways. Together these findings highlight the role of ferroptosis as an emerging concept in cancer biology and an attractive target for precision cancer medicine discovery.

1. INTRODUCTION

Multicellular organisms must balance cell proliferation and cell death to ensure tissue homeostasis and prevent the onset of neoplastic diseases (Evan & Vousden 2001, Hanahan & Weinberg 2011). The elimination of damaged and unnecessary cells occurs through the activation of apoptosis or one of several regulated nonapoptotic cell death pathways, such as necroptosis, pyroptosis, and ferroptosis (Galluzzi et al. 2018). Apoptosis has a clear role in tumor suppression and in the lethal effects of anticancer drugs (Delbridge et al. 2012; Johnstone et al. 2002; Korsmeyer 1999; Ni Chonghaile et al. 2011; Sarosiek et al. 2013, 2017). By contrast, the role of nonapoptotic cell death pathways in these processes is just beginning to emerge (Brumatti et al. 2016, Jiang et al. 2015, Koo et al. 2015, Y. Wang et al. 2017). In this review, we focus on the nonapoptotic cell death process of ferroptosis (Cao & Dixon 2016, Dixon et al. 2012, Gao & Jiang 2017, Stockwell et al. 2017, Yang & Stockwell 2016). We describe three hallmarks of ferroptosis, discuss how this process can be activated in cancer cells by investigational and approved drugs, and consider whether this process may contribute to natural tumor suppression.

2. THE HALLMARKS OF FERROPTOSIS

Studies over the past decade have defined a core ferroptotic pathway that is distinct from other forms of regulated cell death (Galluzzi et al. 2018). This process is likely to be relevant to cell death in diverse pathological contexts in humans, as well as in various physiological contexts in diverse animals, plants, and potentially even bacteria (see Conrad et al. 2018). However, ferroptosis was originally recognized as a unique form of cell death in cultured mammalian cancer cells by studying the effects of the small molecule erastin, (1*S*, 3*R*)-RSL3 (hereafter RSL3), and related compounds (**Figure 1**). These compounds were discovered in phenotypic screens for small molecules that are selectively more lethal to engineered human tumor cells overexpressing mutant (oncogenic) *HRAS* (Dolma et al. 2003, Weiwer et al. 2012, Yang & Stockwell 2008). These compounds trigger cell death without classic morphological or biochemical features of apoptosis, such as chromatin margination or caspase activation (Dolma et al. 2003, Yagoda et al. 2007, Yang & Stockwell 2008). Moreover, cell death induced by these compounds is morphologically, biochemically, and genetically distinct from apoptosis, classic necrosis, and other forms of nonapoptotic cell death (Dixon et al. 2012). By contrast, a defining feature of ferroptosis is the requirement for iron and the accumulation of reactive oxygen species (ROS): Both iron chelators and lipophilic antioxidants potently inhibit lipid ROS accumulation and cell death in response to erastin, RSL3, and other compounds (Dixon et al. 2012, Yagoda et al. 2007, Yang & Stockwell 2008). From these and other studies, we suggest that there are three core hallmarks of ferroptosis (**Figure 2**).

2.1. Oxidation of Polyunsaturated Fatty Acid–Containing Phospholipids

The membranes of mammalian cells are rich in glycerophospholipids [hereafter simply phospholipids (PLs)] acylated with at least one polyunsaturated fatty acid (PUFA) chain. PUFAs of various chain lengths (C18 or higher) and degrees of unsaturation (e.g., C18:3, C20:4, C22:5) can be incorporated into PLs with different head groups [e.g., phosphatidylinositol, phosphatidylcholine, or phosphatidylethanolamine (PE)], yielding a plethora of unique phospholipid species, potentially numbering in the thousands (Magtanong et al. 2016). The importance of PUFA-containing PLs in ferroptosis is underscored by the observation that deletion of genes (e.g., *ACSL4*, *LPCAT3*) required for the activation or incorporation of activated PUFAs into membrane PLs prevents ferroptosis (Dixon et al. 2015, Doll et al. 2017, Kagan et al. 2017, Yuan et al. 2016b); **Figure 3** highlights these and other key proteins involved in ferroptosis. This result also indicates that

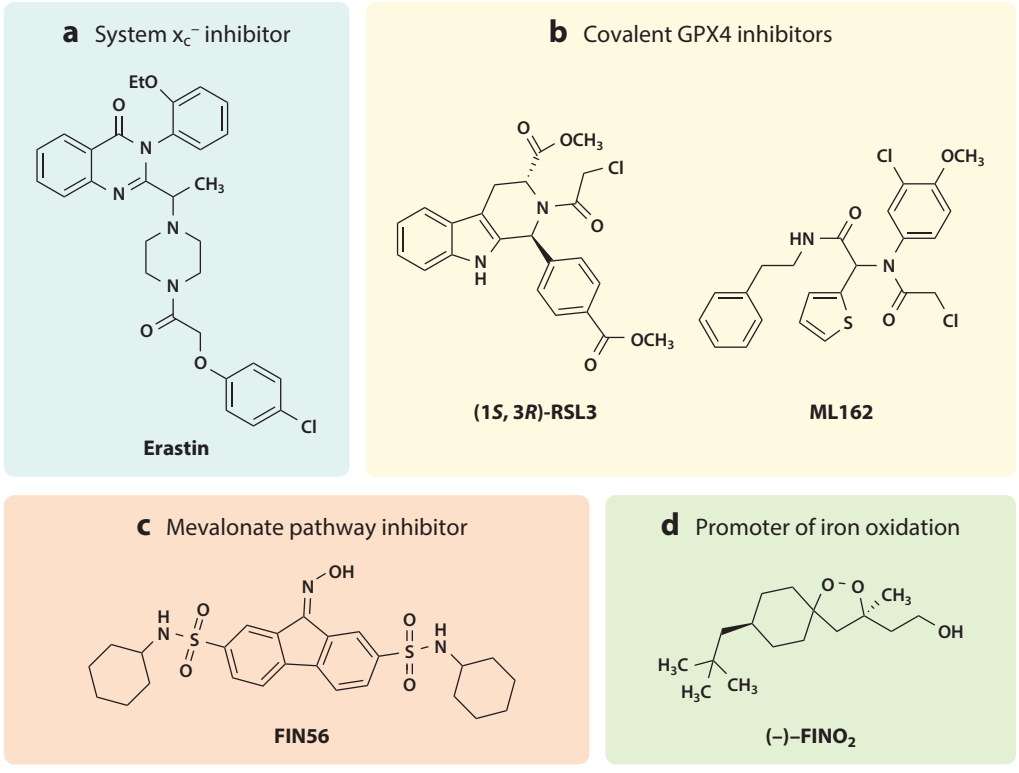

Figure 1

Compounds that induce ferroptosis. Structures are shown for four classes of ferroptosis-inducing (FIN) compounds.

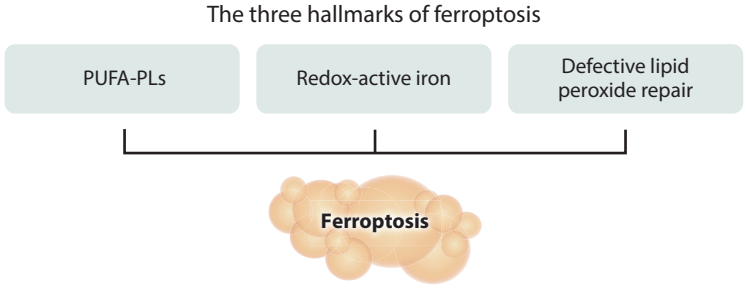

Figure 2

The hallmarks of ferroptosis. Three hallmark features define cancer cell sensitivity to ferroptosis: the presence of oxidizable phospholipids (PLs) acylated with polyunsaturated fatty acids (PUFA-PLs), the presence of redox-active iron, and defective or inhibited lipid peroxide repair. All three hallmark features are required for the execution of ferroptosis.

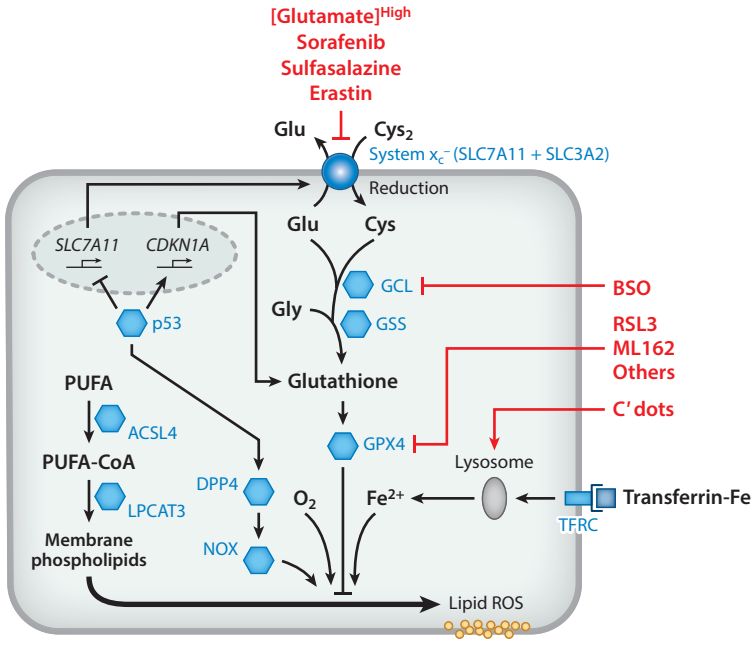

Figure 3

The ferroptosis pathway. Illustration of key pathways and processes relevant to the initiation and execution of ferroptosis. Key protein regulators of ferroptosis are shown in blue, while several inducers of ferroptosis are highlighted in red. Abbreviations: BSO, buthionine sulfoximine; CoA, coenzyme A; Cys, cysteine; Cys_2, cystine; Glu, glutamate; Gly, glycine; NOX, NADPH oxidase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RSL3, (1*S*, 3*R*)-RSL3; TFRC, transferrin receptor.

free PUFAs are unlikely to promote ferroptosis; they must be activated and incorporated into membrane PLs to participate in this lethal process.

Unlike saturated fatty acids and monounsaturated fatty acids, the bis-allylic hydrogen atoms present within PUFAs are highly susceptible to free radical or enzyme-mediated oxidation (Gaschler & Stockwell 2017). PUFA oxidation is essential for the execution of ferroptosis in response to diverse stimuli and can be inhibited by feeding cells deuterated PUFAs that are less susceptible to oxidation (Skouta et al. 2014, Yang et al. 2016). The oxidation of arachidonoyl (C20:4) or adrenoyl (C22:4) fatty acyl chains in the context of PE PLs (i.e., PUFA-PE) is specifically linked the execution of ferroptosis in at least some cell types (Kagan et al. 2017, Wenzel et al. 2017). At the molecular level, how oxidation of membrane PUFA-PEs and other species leads to cell death is still unclear. This could involve formation of a structured lipid pore, similar to the proteinaceous pores observed in necroptosis and pyroptosis (Kayagaki et al. 2015, Y. Wang et al. 2017). Alternatively, PUFA-PL oxidation could lead to membrane thinning and aberrant membrane curvature, which could drive a local vicious cycle, leading to pore formation (Agmon et al. 2018). Thus, the first hallmark of ferroptosis is the availability of PUFA-PLs at a sufficient concentration to promote cell death.

2.2. Redox-Active Iron

As implied by the name, ferroptosis is an iron-dependent form of cell death. Mechanistically, free iron or iron-containing lipoxygenase enzymes are responsible for oxidizing membrane PUFAs,

potentially leading to the formation of lipid ROS. The presence of sufficient free intracellular iron and the presence of membrane PL-PUFAs are both prerequisites for the execution of ferroptosis. Thus, iron chelators such as deferoxamine and ciclopirox block ferroptotic death by interfering with the generation of oxidized lipid species (Stockwell et al. 2017). Iron is imported into the cell as iron-transferrin complexes, a process that can be significantly upregulated in breast, ovarian, and other cancers (Basuli et al. 2017, Torti & Torti 2013). Downregulation of transferrin receptor–mediated import of transferrin-iron complexes suppresses ferroptosis, most likely by limiting the uptake of iron (Gao et al. 2015, 2016; Torii et al. 2016; Yang & Stockwell 2008).

Internalized transferrin receptor/transferrin-iron complexes localize to the lysosome. In response to the acidic conditions of the lysosome, iron is liberated from these complexes and exported into the cytosol. There, the majority of iron is stored within ferritin nanocages, from which it can be liberated via NCOA4-dependent ferritinophagy (Mancias et al. 2014). Silencing of *NCOA4* inhibits ferroptosis in HT-1080 fibrosarcoma, Panc-1 pancreatic cancer, and other cell types, presumably by reducing the intracellular free iron pools (Gao et al. 2016, Hou et al. 2016). The expression of proteins involved in the utilization of iron for iron-sulfur cluster biogenesis, including CDGSH iron-sulfur domain 1 and the cysteine desulfurase NFS1, reduces ferroptosis sensitivity, presumably by limiting the accumulation of free, redox-active iron (Alvarez et al. 2017, Yuan et al. 2016a). Iron dependency therefore constitutes the second hallmark of ferroptosis sensitivity.

2.3. Loss of Lipid Peroxide Repair

Given the potential toxicity of iron-catalyzed PL-PUFA oxidation, this process is normally maintained under tight control. A selenoenzyme, the (reduced) glutathione (GSH)-dependent lipid hydroperoxidase glutathione peroxidase 4 (GPX4), is specialized for the reduction of lipid hydroperoxides to lipid alcohols in membrane environments (Ursini et al. 1985). GPX4 is one of 25 selenoproteins expressed in human cells and the only one that appears essential for normal mammalian development (Ingold et al. 2018). Inactivation of this enzyme is sufficient to induce ferroptosis in many cell types, and deletion of the *Gpx4* gene is lethal to mice (Ingold et al. 2018, Seiler et al. 2008, Yang et al. 2014). While GPX4 appears to be the dominant antioxidant enzyme acting to prevent the accumulation of toxic lipid ROS during ferroptosis, small-molecule lipophilic antioxidants, including vitamin E (α-tocopherol) and coenzyme Q_{10} (CoQ_{10}), also help detoxify membrane lipid ROS. Remarkably, certain cancer cells can survive and proliferate following deletion of *GPX4* (Viswanathan et al. 2017). This could be due to the compensatory activity of alternative endogenous antioxidant pathways, the upregulation of other (unknown) enzymes or metabolites capable of suppressing lipid peroxidation, or the existence of cancer cells with low endogenous levels of oxidizable PL-PUFAs; this observation suggests that not all cells are intrinsically susceptible to GPX4 inactivation–mediated ferroptosis. Like endogenous antioxidants, exogenous lipophilic antioxidants [e.g., ferrostatin-1, liproxstatin-1 (Dixon et al. 2012, Friedmann Angeli et al. 2014)] potently inhibit ferroptosis by preventing the propagation of oxidative damage within the membrane (Zilka et al. 2017). In short, the third hallmark of ferroptosis is the loss of the repair system for eliminating lipid hydroperoxides from PUFA-PLs.

3. STRATEGIES TO INDUCE FERROPTOSIS IN CANCER CELLS

The ferroptosis pathway described above may offer new targets for the development of anticancer therapies. Inducing ferroptosis requires the ability to create an imbalance between lipid

hydroperoxide detoxification and iron-dependent lipid ROS accumulation. Conditions that push this balance in favor of lipid hydroperoxide accumulation stimulate ferroptosis. Several strategies designed to promote this imbalance are being pursued in connection with cancer therapy in preclinical settings. Moreover, as described below, several agents already in clinical use may serendipitously induce this imbalance as part of their mechanism of action in vivo.

3.1. Increasing Intracellular Iron Levels

Iron is essential for the execution of ferroptosis. Exposure to high levels of extracellular iron may be sufficient alone to induce ferroptosis in some cells. Treatment with small (~6 nm) surface-functionalized poly(ethylene glycol)-coated (PEGylated) silica nanoparticles (C′ dots) can induce ferroptosis in tumor xenografts; this process correlates with the ability of these particles to transport (load) iron into cells (Kim et al. 2016). Intracellular iron loading may induce ferroptosis due to the relative depletion of amino acids (e.g., cystine) that are otherwise necessary to inhibit ferroptosis in some cell lines (Kim et al. 2016). C′ dots are already used in humans for imaging applications, and it is conceivable that that these agents could be repurposed as ferroptosis-inducing anticancer treatments. Since exogenous iron (e.g., ferric ammonium citrate) does not appear capable of inducing ferroptosis in all cell types, at least in vitro (Dixon et al. 2012), the method of iron delivery, the site of intracellular accumulation, and cell lineage could all impact whether a given iron treatment is able to induce ferroptosis in a specific context.

3.2. Inhibiting GPX4 Expression

GPX4 is a selenoprotein. The synthesis of the active site selenocysteine (Sec) requires specialized machinery to incorporate selenium into this amino acid and then conjugate Sec onto its transfer RNA (tRNA). The Sec-charged tRNA is isopentenylated using a substrate (isopentenyl pyrophosphate) derived from the mevalonate pathway, and this modification is essential for efficient translational decoding of this tRNA (Warner et al. 2000). Statins are potent inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, a key enzyme in the mevalonate pathway. Thus, the loss of GPX4 protein and the enhanced sensitivity to ferroptosis observed in some cancer cells treated with statins (e.g., fluvastatin) could be due to inhibition of GPX4 synthesis (Viswanathan et al. 2017). Crucially, statin treatment is tolerated in humans and is widely used to lower cholesterol levels. Intriguingly, known side effects of statin treatment (e.g., myopathy) are plausibly attributable to reduced GPX4 levels in some tissues (Moosmann & Behl 2004), and statin consumption in humans is linked to decreased incidence of various cancers, including colorectal, melanoma, and prostate cancers (Demierre et al. 2005). While speculative, the beneficial effects of statins observed in certain patients could in part be attributable to reduced levels of GPX4 and the induction of ferroptosis. Alternatively or in parallel, statin treatment could deplete CoQ_{10} (another product of the mevalonate pathway) and reduce the levels of this key membrane antioxidant (Qi et al. 2010).

3.3. Inhibition of GPX4 Enzymatic Activity and Expression

GPX4 uses reduced GSH as an enzyme cosubstrate in the conversion of lipid hydroperoxides to lipid alcohol. RSL3, ML162 (**Figure 1**), and related compounds directly inhibit GPX4 function via covalent modification, thereby triggering ferroptosis without altering intracellular GSH levels (Shimada et al. 2016b, Weiwer et al. 2012, Yang et al. 2014). The structurally distinct ferroptosis inducers FIN56 and (–)–$FINO_2$ (**Figure 1**) also trigger ferroptosis (Abrams et al. 2016, Gaschler

et al. 2018, Shimada et al. 2016b). Like statins, FIN56 acts in part to inhibit the mevalonate pathway and therefore the synthesis of the Sec-containing GPX4 protein (and CoQ_{10}), while $FINO_2$ causes iron oxidation and loss of GPX4 enzymatic activity without depleting GPX4 protein (Abrams et al. 2016, Gaschler et al. 2018, Shimada et al. 2016b).

Collectively, these GPX4-inhibiting agents are useful small-molecule tool compounds but have not been investigated for in vivo efficacy. By contrast, altretamine is a novel candidate GPX4 inhibitor already approved for use in treating cancer, but whose mechanism of action has long been mysterious (Woo et al. 2015). As with statins, it is conceivable that some of the clinical benefits observed with altretamine could be attributable to the inhibition of GPX4 and induction of ferroptosis, but this has not been examined mechanistically in vivo.

3.4. Indirect Inhibition of GPX4 Activity: Inhibition of Glutathione Synthesis

Since GPX4 requires GSH as a cofactor, targeted depletion of this metabolite or inhibition of de novo GSH synthesis should result in inactivation of all GSH-dependent enzymes. Decades ago, it was shown that depleting GSH could enhance the sensitivity of cancer cells to radiation and alkylating chemotherapeutics (Bump et al. 1982, Somfai-Relle et al. 1984). A small-molecule inhibitor of de novo GSH synthesis, buthionine sulfoximine (BSO) (Griffith & Meister 1979), was subsequently evaluated in human clinical trials. It was found that BSO (in combination with melphalan) was tolerated in humans with moderate (~60%) GSH depletion in normal peripheral mononuclear cells and substantial (up to 90%) GSH depletion in cancer cells with manageable systemic toxicities (Bailey et al. 1994, 1997; Calvert et al. 1998). Clinical studies continue to investigate the utility of BSO-mediated GSH depletion in cancer therapy (Villablanca et al. 2016), but to date, there is not a strong clinical rationale for the addition of BSO to standard chemotherapy routines. It is unclear whether any of the clinical responses that have been observed in previous studies are due to the induction of ferroptosis or due to increased activity of the primary chemotherapeutic agent (e.g., due to reduced drug detoxification in low-GSH cells).

In vitro, BSO is an unexpectedly weak inducer of ferroptosis alone but can strongly potentiate the effects of agents targeting system x_c^- (see below) or GPX4. This may be because system x_c^-–dependent cystine/cysteine shuttle activity can contribute independently to protection from ferroptosis (Banjac et al. 2008), because GSH-independent antioxidant systems (e.g., thioredoxin) play a coequal antioxidant role in some cancer cells (Harris et al. 2015), or because certain pools of GSH within the cell are not effectively depleted by BSO treatment. Thus, it is possible that agents that conjugate directly to GSH will prove more effective at inactivating key pools of GSH within the cell that prevent ferroptosis. For example, the cytotoxic compound APR-246 (PRIMA-1) was found to induce ferroptosis-like cell death through conjugation to GSH and depletion of this molecule (Liu et al. 2017). Other approaches to deplete GSH indirectly by hyperactiving GSH-dependent metabolic processes, such as by incubating cells in high concentrations of vitamin C, might work in some cancers, but whether this results in the induction of ferroptosis is not known (Schoenfeld et al. 2017, Yun et al. 2015).

3.5. Indirect Inhibition of GPX4 Activity: Inhibition of Cystine Import

Within cells, the amino acid cysteine is rate limiting for the biosynthesis of GSH. In some cells, cysteine can be synthesized from methionine via transsulfuration, but in many proliferative cells cysteine is obtained from extracellular cystine (the cysteine disulfide is known as cystine). Cystine can be transported into cells via the dedicated plasma membrane antiporter designated system x_c^-. System x_c^- is composed of the regulatory subunit 4F2 heavy chain (known as 4F2hc, CD98, or

SLC3A2) and the transport subunit xCT (encoded by the gene *SLC7A11*) (Sato et al. 1999). At the plasma membrane, system x_c^- imports cystine in exchange for glutamate in an ATP-independent manner. Indeed, when extracellular glutamate levels are elevated, this can prevent amino acid exchange, block cystine import, and trigger ferroptosis in cancer cells (Dixon et al. 2012).

The levels of xCT transcript and/or transport activity are elevated in some cancers, including colon, liver, breast, and gastric cancers, indicating that these cells may exhibit a heightened dependency upon cystine uptake for survival (Ishimoto et al. 2011, Jiang et al. 2015, Timmerman et al. 2013). Sulfasalazine (SAS), a medicine used to treat arthritis, is a low-potency inhibitor of system x_c^- that has demonstrated activity in preclinical cancer models but must be used at high concentrations (Gout et al. 2001). SAS was tested in a phase I/II trial of high-grade glioma and was found to have no therapeutic effect (Robe et al. 2009), possibly because it cannot pass the blood-brain barrier and is metabolically unstable. A more recent clinical investigation of SAS in patients with gastric adenocarcinoma appeared more promising (Shitara et al. 2017), but larger numbers of subjects and a randomized trial design will be required to draw firm conclusions.

Unlike SAS, erastin and derivatives thereof are potent inhibitors of system x_c^-–mediated cystine import ($EC_{50} < 10\ \mu M$) (Dixon et al. 2012, 2014). While the original compound erastin was not suitable for use in animal studies, improved erastin derivatives with better metabolic stability may enable this mechanism to be accessed in vivo (Larraufie et al. 2015, Yang et al. 2014). Additionally, some evidence suggests that the multikinase inhibitor sorafenib can induce ferroptosis by inhibiting system x_c^- function (Dixon et al. 2014, Lachaier et al. 2014, Louandre et al. 2013). Interestingly, sorafenib treatment induces an accumulation of ROS in vivo, and this may be predictive of patient responses (Coriat et al. 2012), consistent with the notion that the induction of ferroptosis could explain a portion of the existing effects of sorafenib in patients. Of note, deletion of *Slc7a11* in mice is compatible with normal development and relatively mild phenotypes in adults (Chintala et al. 2005, Sato et al. 2005), suggesting that system x_c^- function is largely dispensable for development. If cancer cells become more dependent upon the function of this transporter (see above), there may be a substantial therapeutic window available to inhibit system x_c^- function to selectively target cancer cells in vivo.

3.6. Indirect Inhibition of GPX4 Activity: Depletion of Extracellular Cystine

In addition to blocking cystine uptake, a novel approach to inhibiting GSH synthesis has been developed that involves degradation of cystine/cysteine within the serum by an engineered enzyme, cyst(e)inase (Cramer et al. 2017). In vitro, cyst(e)inase induces hallmarks of ferroptosis including GSH depletion and ROS accumulation. In vivo, treatment with cyst(e)inase decreased serum cystine and cysteine levels without overt toxicity to mice. This enzyme could also arrest the growth of prostate and breast xenograft tumors and prolong survival in a mouse model of chronic lymphocytic leukemia (Cramer et al. 2017). This enzyme has also shown efficacy in epidermal growth factor receptor (EGFR)-mutant NCI-NH1650 xenografts in vivo (Poursaitidis et al. 2017), suggesting that it could emerge as a standard approach to depleting serum cystine and inducing ferroptosis.

4. OTHER TARGETABLE PATHWAYS MODULATING FERROPTOSIS SENSITIVITY

Ferroptosis occurs when lipid hydroperoxide detoxification pathway activity is reduced to such an extent that it becomes insufficient to restrain iron-dependent membrane PUFA oxidation and

toxic lipid ROS accumulation. Consequently, genes and pathways that regulate GPX4 expression, GPX4 activity, endogenous lipophilic antioxidant pathways, PUFA metabolism, or iron handling emerge as important regulators of ferroptosis sensitivity. Interestingly, many of these processes are differentially regulated in cancer versus normal cells and can therefore alter cancer cell sensitivity to ferroptosis. In this section, we describe several of these direct regulatory links and consider how alteration of these processes within the tumor context could contribute to differential ferroptosis sensitivity.

4.1. The RAS/MAPK Pathway

Ferroptosis can be selectively activated in engineered oncogenic $HRAS^{V12}$-expressing cancer cells (Dolma et al. 2003, Yagoda et al. 2007). Additionally, silencing of oncogenic KRAS expression in KRAS-mutant Calu-1 cells significantly reduces the lethality of erastin (Yagoda et al. 2007). RAS activation could enhance ferroptosis sensitivity by increasing the expression of transferrin receptor and intracellular iron levels (Yang & Stockwell 2008). However, when sensitivity to compound-induced ferroptosis was surveyed across a broad panel of cancer cell lines, no correlation was observed between RAS mutation status and the potency of erastin (Yang et al. 2014). In fact, in rhabdomyosarcoma cells, ectopic expression of oncogenic RAS actually increases resistance to erastin-induced ferroptosis (Schott et al. 2015). A possible reconciliation of these findings is that, within a given cancer cell, oncogenic RAS pathway activation can alter sensitivity to this process, but that RAS pathway activity alone is not the sole determinant of ferroptosis sensitivity.

It is likely that the above concept of lineage-specific and mutation-specific responses extends to the role of the mitogen-activated protein kinase (MAPK) pathway. For example, in melanoma, differences between cells in ferroptosis sensitivity do not correlate with differences in activation of the MAPK pathway (Tsoi et al. 2018). By contrast, in lung cancer cells the situation is different. Activating mutations or overexpression of EGFR are observed in lung and other cancers. EGFR is a receptor tyrosine kinase (RTK) that transduces signals through the MAPK and other pathways. Human mammary epithelial cells engineered to express an oncogenic form of EGFR containing a deletion of four amino acids (E^{746}–A^{750}) were highly susceptible to the induction of ferroptosis in response to cystine deprivation (Poursaitidis et al. 2017). Oncogenic mutations in *KRAS*, *BRAF*, and *PIK3CA* also sensitized to cystine deprivation–induced death. Interestingly, NSCLC cell lines with the highest relative MAPK activation were most sensitive to cystine deprivation–induced ferroptosis (Poursaitidis et al. 2017), consistent with the original observation that activation of the RAS/MAPK pathway sensitizes to ferroptosis within individual cell lines (Dolma et al. 2003, Yagoda et al. 2007). How EGFR or other signals promote sensitivity to ferroptosis and whether mutational signatures or functional assays of the RTK-RAS/MAPK pathway are sufficient to predict the sensitivity of a given cancer cell to ferroptosis clinically are not known.

4.2. The NRF2 Pathway

Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, also known as NRF2) is a master regulator of the cellular antioxidant and xenobiotic detoxification response that is negatively regulated by the E3 ubiquitin ligase KEAP1 (Jaramillo & Zhang 2013). Mutations in KEAP1 are observed in many tumors and NRF2 is essential for growth and survival of cancers harboring oncogenic KRAS and other mutations (Chio et al. 2016, DeNicola et al. 2011, Jaramillo & Zhang 2013,

Lien et al. 2016, Romero et al. 2017). NRF2 positively regulates several genes that are relevant to ferroptosis, including those involved in GSH biosynthesis [e.g., *SLC7A11*, *GCLC*, *GCLM* (Chen et al. 2017, Wakabayashi et al. 2003)] and iron homeostasis [e.g., *HMOX1*, *FTH1*, *FPN* (Kerins & Ooi 2018)]. In hepatocellular cancers, genetic silencing of NRF2 enhances sensitivity to ferroptotic agents (e.g., BSO, erastin, sorafenib) (Sun et al. 2016). Likewise, in head and neck cancer cells, NRF2 expression decreases sensitivity to the ferroptosis-inducing activity of artesunate (Roh et al. 2017). While the mechanism of NRF2-mediated resistance to ferroptosis is not definitely established in these models, it may involve regulation of *SLC7A11* expression itself, alterations in iron levels, or upregulation of enzymes that detoxify end products of lipid peroxidation.

Since NRF2 controls the expression of a battery of phase II xenobiotic detoxification genes, activation of this pathway could also promote cancer cell resistance to ferroptosis by detoxifying and exporting from the cell compounds that would otherwise induce this process. A general prediction of these findings is that tumor cells with high levels of unimpeded NRF2 pathway activation (e.g., due to KEAP1 mutations) are likely to be less sensitive to ferroptosis. These considerations also suggest one possible explanation for the lack of correlation between RAS mutation status and ferroptosis sensitivity: The co-occurrence of RAS pathway mutations with KEAP1 mutations may yield opposing effects on ferroptosis sensitivity. More speculatively, treatment with agents that inhibit the function of the NRF2 pathway [e.g., ML385 (Singh et al. 2016)] could be combined with ferroptosis inducers.

The NRF2 pathway does not operate in isolation within the cell, and links are emerging between NRF2 and classic tumor suppressors in the regulation of ferroptosis sensitivity. The tumor suppressor ARF (*CDKN2A*) has recently been identified as a binding partner for NRF2 that impacts ferroptosis sensitivity (Chen et al. 2017). ARF represses NRF2 transcription upregulation of *SLC7A11* and other antioxidant genes by interfering with CBP-dependent NRF2 acetylation and, thus, favors the induction of ferroptosis in response to certain stimuli (e.g., tert-butyl hydroperoxide) (Chen et al. 2017).

Another cell cycle regulatory protein, the cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ (encoded by *CDKN1A*), can compete with KEAP1 for binding to NRF2, therefore stabilizing NRF2 and promoting an antioxidant response in colorectal cancer cells and mice (Chen et al. 2009). ARF and p21 could thus have opposing roles in the regulation of NRF2 function and, hence, ferroptosis. Such interactions may be specific to cell type, as deletion of *CDKN1A* does not compromise the activation of the NRF2 pathway in all cancer cells (Tarangelo et al. 2018).

4.3. Autophagy

Inhibition of system x_c^- can lead to increased autophagy and ferritinophagy, and inhibition of autolysosomal function using bafilomycin A_1 or chloroquine partially inhibits ferroptosis by reducing the turnover of iron-containing ferritin molecules and the release of iron (Gao et al. 2016, Hou et al. 2016, Torii et al. 2016). Thus, autophagy can promote ferroptosis indirectly by making available to the cell the free iron necessary for lipid peroxidation and the execution of cell death. That said, it does not appear that autolysosomal digestion of cellular contents (e.g., membranes) contributes directly to the execution of ferroptosis per se, as in *Drosophila melanogaster* (Anding & Baehrecke 2015). Cells lacking key autophagy genes (e.g., *ATG5*, *ATG7*) remain competent to execute ferroptosis, albeit with reduced sensitivity in some cases (Gao et al. 2016, Hou et al. 2016). Nonetheless, enhanced autophagy could promote ferroptosis by increasing free intracellular iron levels.

5. FERROPTOSIS-SENSITIVE CELL STATES

5.1. Mesenchymal State

Cells can adopt different states depending on variable patterns of gene expression. These states may predispose to ferroptosis and suggest areas where the application of proferroptotic therapies are most likely to find useful application. For example, the mesenchymal state is associated with enhanced sensitivity to direct (e.g., RSL3) and indirect (e.g., statin) GPX4 inhibition (Viswanathan et al. 2017). Several different pathways can promote the mesenchymal state, and one pathway, driven by the transcription factor ZEB1, enhances ferroptosis sensitivity, possibly through effects on lipid metabolism (Viswanathan et al. 2017).

5.2. Drug-Tolerant Persisters

Cancer cells with defined genetic vulnerabilities can be highly sensitive to targeted therapies, but close inspection often reveals a small fraction of drug-tolerant persister (DTP) cells that can evade the lethal effects of these treatments (Inde & Dixon 2018). The DTP state is defined by a unique epigenetic signature and altered expression of key signaling and metabolic genes, although the precise molecules responsible for conferring drug tolerance are rarely clear (Sharma et al. 2010). The existence of DTPs within larger tumor cell populations is therapeutically problematic and could provide a substrate for the emergence of frank drug-resistant clones following multiple rounds of drug selection (Hata et al. 2016). Interestingly, several different isolated DTP populations exhibit enhanced sensitivity to ferroptosis-inducing agents like the GPX4 inhibitor ML162 (Hangauer et al. 2017). Mechanistically, these DTPs appear to have reduced levels of both GSH and NADPH, possibly due to the downregulation of an NRF2-dependent antioxidant gene expression program (Hangauer et al. 2017). This renders these DTPs less able to handle proferroptotic stimuli such as GPX4 inhibition. The selective induction of ferroptosis in persister cells may provide a route to eliminate certain residual tumor cells in concert with other therapies (Hangauer et al. 2017).

5.3. Dedifferentiated Cells

The differentiation status of melanoma cells has been linked to ferroptosis sensitivity. Melanoma arises from melanocytes, which are multipotent progenitors of the neural crest, and these cancer cells can adopt gene signatures that are consistent with at least four different differentiation states, from undifferentiated to more differentiated (Tsoi et al. 2018). Certain melanoma cells exist in a mostly dedifferentiated state normally, while others acquire this state in response to treatment with targeted inhibitors of the MAPK pathway or to immunotherapy. Regardless, this dedifferentiated state is associated with greater sensitivity to system x_c^- inhibitors (i.e., erastin) and to direct GPX4 inhibitors (e.g., RSL3) (Tsoi et al. 2018). In line with observations made in DTP cells, long-term treatment of melanoma cells with a BRAF inhibitor can increase sensitivity to ferroptosis-inducing agents, implying that this alteration is a result of gene expression changes within the cell downstream of BRAF inhibition. As with DTPs, the less differentiated, ferroptosis-sensitive state is associated with reduced levels of basal GSH, likely accounting for their greater sensitivity (Tsoi et al. 2018). Thus, while more differentiated melanoma cells are highly sensitive to BRAF inhibitors and immunotherapy, less differentiated or dedifferentiated cells acquire a greater sensitivity to ferroptosis that could be exploited to selectively eliminate these cells. Whether dedifferentiation is commonly associated with ferroptosis sensitivity in other cell lineages remains to be tested,

but it is notable that the conversion of brain astroglia cells to neurons may require evasion of a ferroptotic checkpoint, consistent with increased sensitivity to ferroptosis when cell states are in flux (Gascón et al. 2016).

5.4. Detached Cells

Solid tumor cells are grown in contact with the surrounding extracellular matrix, which provides key signaling inputs through integrin and other signaling pathways. In mammary epithelial and breast carcinoma cells, loss of α6β4 integrin, but not other integrins, specifically sensitizes cells to ferroptosis induced by erastin (Brown et al. 2017). α6β4 integrin is especially important to maintain viability when normally adherent breast cancer cell lines cells are cultured under matrix-detached (i.e., nonadherent) conditions. The spontaneous death of matrix-detached, α6β4-null cells can be partially suppressed by cotreatment with ferrostatin-1, deferoxamine, and other ferroptosis inhibitors (Brown et al. 2017). Mechanistically, α6β4 expression inhibits ferroptosis under detached conditions by (*a*) activating a SRC-signal transducer and activator of transcription 3 (STAT3) pathway that suppresses the expression of ACSL4 and (*b*) increasing the expression of GPX4 through an unknown pathway. Thus, when the expression or function of α6β4, SRC, or STAT3 is disrupted, increased ACSL4 and decreased GPX4 expression presumably conspire to promote ferroptosis. Of note, cell death under matrix-detached conditions is also partially suppressed by a pan-caspase inhibitor, Z-VAD-FMK, indicating that either a ferroptotic or an apoptotic mechanism may eliminate matrix-detached cells. Further investigation has revealed that cells in the detached state can exist either in clusters or as single cells, with the former being highly sensitive to ferroptosis and the latter to apoptosis (Brown et al. 2018). Cell clustering was associated with higher levels of lipid ROS accumulation and was dependent on the cell surface protein nectin cell adhesion molecule 4 (NECTIN4, also known as PVLR4) (Brown et al. 2018). How NECTIN4-mediated cell clustering promotes ferroptosis in detached cells lacking α6β4 integrin remains to be defined. However, these studies indicate that cell detachment itself is a physiological condition that may normally predispose cancer cells to ferroptosis.

5.5. Cancer Stem Cells

A variant of the natural product salinomycin, named ironomycin (or AM5), is selectively lethal to $CD44^{High}/CD24^{Low}$ breast cancer stem cells and patient-derived xenograft tumors in mice through a mechanism involving the lysosomal iron binding, leading to enhanced lysosomal concentration of the iron storage protein ferritin and subsequent iron release, lysosomal leakage, enhanced intracellular ROS accumulation, and cell death, which could be partially prevented by cotreatment with ferrostatin-1 (Mai et al. 2017). Interestingly, these breast cancer stem cells were characterized by higher transferrin receptor and intracellular iron levels, which could explain their predisposition to further increases in iron levels and sensitivity to oxidative cell death (Mai et al. 2017). Glioblastoma cancer stem cells also exhibit increased iron uptake (Schonberg et al. 2015), raising the possibility that this is a shared feature of several diseases. This may not be a universal response, however, as freshly isolated breast cancer stem cells exhibit higher expression of GSH metabolic enzymes (e.g., *GCLM*) and greater resistance to oxidative stress (Diehn et al. 2009). Further research is required to define what features of cancer stem cells render them more or less susceptible to ferroptosis.

6. FERROPTOSIS AND TUMOR SUPPRESSION

Ferroptosis is constantly suppressed by an intracellular antioxidant network involving GPX4, CoQ_{10}, and other molecules. Ferroptosis can be initiated by signals such as cystine deprivation that lead directly to depletion of a metabolite (i.e., GSH) that prevents lipid ROS accumulation. Unlike other forms of regulated cell death, the execution of ferroptosis is not known to require transcriptional upregulation of a specific prodeath gene or posttranslational activation of a specific prodeath protein (e.g., via proteolytic cleavage or phosphorylation) (Cao & Dixon 2016, Stockwell et al. 2017). The apparent absence of complex regulatory control over ferroptosis could make it possible to regulate cell viability by inhibiting the activity of a single metabolic pathway or the accumulation of a specific metabolite (e.g., cystine, GSH), thereby suppressing neoplastic growth.

A link between ferroptosis and tumor suppression first emerged from studies of a p53 mutant that cannot be acetylated at three key lysine residues (i.e., the 3KR mutant). This mutant form of p53 is unable to induce apoptosis, cell cycle arrest, or senescence, and yet it retains the ability to suppress tumor formation in mice (Jiang et al. 2015, Li et al. 2012). Interestingly, this mutant sensitized cancer cells to ferroptosis, an effect traced to the direct repression of *SLC7A11* expression (Jiang et al. 2015). Subsequent work identified a fourth lysine residue (K98) that, when mutated together with the three previously identified lysine residues, resulted in a p53 protein that was unable to induce ferroptosis or suppress xenograft tumor growth in mice (Wang et al. 2016). The correlation between the expression of certain acetylation-defective p53 variants (i.e., 3KR), the induction of ferroptosis, and the suppression of tumor growth in mice suggests that these processes are related. Additional support for the model that p53 promotes ferroptosis comes from the study of a natural p53 polymorphic variant (P47S). The P47S mutation partially compromises the tumor-suppressive abilities of p53, leading to higher tumor incidence in mice and in humans harboring this mutation (Jennis et al. 2016). In vitro, P47S expression is associated with reduced sensitivity to compound-induced ferroptosis, suggesting that the induction of this process could be normally compromised in vivo (Jennis et al. 2016).

Despite these results, several observations suggest that the role of p53 (and the p53 family) in the regulation of ferroptosis is likely to be complex. In colorectal cancer cells, wild-type p53 inhibits ferroptosis through a posttranslational interaction with the protease DPP4, which enhances membrane lipid peroxidation in a protease-independent manner via interaction with ROS-generating NADPH oxidase (NOX) enzymes (Xie et al. 2017). NOX enzymes were previously implicated in promoting ferroptosis, although the role of these enzymes appears to be specific to cell type (Dixon et al. 2012). In several other human and mouse cancer cell lines, p53 stabilization (using the MDM2 inhibitor nutlin-3) significantly reduced sensitivity to ferroptosis, induced by system x_c^- inhibition or cystine deprivation, and this p53-dependent protective effect required transcriptional upregulation of the p53 target gene *CDKN1A* ($p21^{CIP1/WAF1}$) (Tarangelo et al. 2018). In these experiments, p21 stabilization was associated with the conservation of intracellular GSH stores, which presumably prolong survival in response to cystine deprivation (Tarangelo et al. 2018). Of note, the p53-3KR mutant is unable to upregulate p21 expression (Jiang et al. 2015), possibly explaining why this protein yields a phenotype that is distinct from that observed with fully wild-type p53. How p21 expression leads to conservation of intracellular GSH remains unclear, but similar p53-/p21-dependent protective effects have been observed in response to other metabolic stresses (Maddocks et al. 2013).

In addition to wild-type p53, expression of ΔNp63α, the major isoform of the p53 relative p63, can suppress ferroptosis and other forms of oxidative stress–induced cell death by increasing the expression of GSH biosynthetic genes, including *SLC7A11* (G.X. Wang et al. 2017). By contrast, oncogenic mutant forms of p53 (e.g., $p53^{R273H}$) can promote sensitivity to ferroptosis-like cell

death (Liu et al. 2017). This mechanism involves binding of mutant p53 to NRF2 and inhibition of NRF2-dependent transactivation of *SLC7A11* and other antioxidant genes that oppose ferroptosis. Currently it is not easy to reconcile these data within a single comprehensive model. One possibility is that p53 and other p53 family members can induce ferroptosis in some contexts and suppress ferroptosis in others, perhaps depending upon the posttranslational modification state of the protein. While complex, this interpretation would be consistent with the ability of p53 to influence other biological processes within the cell in both a positive and negative manner, depending on the situation (Kruiswijk et al. 2015).

7. CONCLUSIONS

Studies to date have elucidated the core hallmarks of ferroptosis sensitivity and have begun to determine how sensitivity to this process is encoded by changes within intracellular metabolic and signaling networks. We propose that ferroptosis sensitivity in general is determined by three hallmarks: (*a*) the availability of redox-active iron, (*b*) the availability of PUFA-PL substrates that are chemically competent to undergo peroxidation, and (*c*) the loss of the lipid repair system of the cell that normally prevents accumulation of these lethal PUFA-PL peroxide species. Just as the genesis of human cancers requires that all of the hallmarks of cancer be activated (Hanahan & Weinberg 2011), the execution of ferroptosis requires the activation of all three of these ferroptosis hallmarks.

An important concept that has emerged from existing research is that—unlike apoptosis and other forms of regulated nonapoptotic cell death—ferroptosis is not defined by the utilization of specific prodeath effector proteins [e.g., caspases, MLKL, gasdermin D (Galluzzi et al. 2018)]. Rather, ferroptosis emerges from the imbalance between cellular metabolic processes (e.g., lipid metabolism, iron handling) that are themselves essential for the growth and proliferation of the cell (Cao & Dixon 2016, Green & Victor 2012). Ferroptosis sensitivity can be altered by the expression and activity of proteins and pathways controlling the levels, transport, storage, and metabolism of iron, PUFAs, cystine, cysteine, GSH, glutamine, and selenium (Stockwell et al. 2017). This may explain why ferroptosis sensitivity can vary so widely between different cell types and even between the same cell type forced into different cell states, each of which is likely defined by a unique pattern of expression for proteins involved iron, lipid, and antioxidant processes.

As outlined above, several existing agents can induce ferroptosis and are also known to cause tumor cell death in vivo. Whether these two properties are related (i.e., whether the induction of ferroptosis is important for the clinical efficacy of these agents) remains to be seen. A key question is which tumor types would most benefit from a proferroptotic therapy. The correlation between ferroptosis sensitivity and tumor genotype (e.g., RAS mutation status) appears weak, and as noted above, the status of various intracellular signaling pathways (e.g., NRF2) and cell states (e.g., DTP versus non-DTP) can impact ferroptosis sensitivity independent of any particular mutation. Thus, alternative approaches to identifying ferroptosis-sensitive cells, lineages, or states will be required.

One promising approach is to identify biochemical markers that predict ferroptosis sensitivity independent of a given genotype. One candidate to recently emerge is the levels of the reduced electron carrier NADPH (Shimada et al. 2016a). NADPH is required to regenerate reduced GSH from oxidized GSSG and also contributes to maintaining the function of GSH-independent antioxidant pathways. NADPH levels may be especially useful in a predictive context, as not all tumor cells remain dependent for survival upon GSH-mediated antioxidant systems but can come to rely upon the GSH-independent, NADPH-dependent thioredoxin pathway (Harris et al. 2015). Validation of NADPH as a marker of ferroptosis sensitivity requires further study, especially in vivo.

Beyond identifying those cells that are sensitive to ferroptosis in vivo, an intriguing question is in which contexts the induction of ferroptosis would be superior to the induction of apoptosis using traditional agents. One possibility is that ferroptosis triggers an immune response in dying tumor cells, as it has been proposed that ferroptosis is part of a necrotic inflammatory cascade in the context of degenerative diseases (Dixon 2017, Giampazolias et al. 2017, Kim et al. 2016, Linkermann et al. 2014). A related question concerns whether it is possible to achieve a large therapeutic index. Doing so may require targeting sensitized cell states (e.g., DTPs, dedifferentiated cells, matrix-detached cells), optimizing pharmacokinetic and pharmacodynamic drug properties of small-molecule agents, and using tumor-selective delivery methods. For example, system x_c^- inhibition is intrinsically tumor selective, since normal cells do not depend on system x_c^-, whereas some tumors become addicted to this cystine-uptake pathway; in contrast, GPX4 is required in the brain and kidney of normal adult mice, suggesting a systemic GPX4 inhibitor may exert undesirable toxicities, at least at the concentrations at which GPX4 is appreciably inhibited in these tissues.

In summary, the three hallmarks of ferroptosis provide a news lens to understand how diverse oncogenic and tumor-suppressive alterations may function in human cancers. Moreover, ferroptosis activation may be a useful strategy for targeting some cancers, overcoming traditional resistance mechanisms to cancer therapies.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank members of the Stockwell and Dixon labs for comments on the manuscript. S.J.D. (1R01GM122923) and B.R.S. (R35CA209896, P01CA087497) are supported by the National Institute of General Medical Sciences and the National Cancer Institute, respectively. S.J.D. is supported by a Damon Runyon-Rachleff Innovation Award.

LITERATURE CITED

Abrams RP, Carroll WL, Woerpel KA. 2016. Five-membered ring peroxide selectively initiates ferroptosis in cancer cells. *ACS Chem. Biol.* 11(5):1305–12

Agmon E, Solon J, Bassereau P, Stockwell BR. 2018. Modeling the effects of lipid peroxidation during ferroptosis on membrane properties. *Sci. Rep.* 8:5155

Alvarez SW, Sviderskiy VO, Terzi EM, Papagiannakopoulos T, Moreira AL, et al. 2017. NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. *Nature* 551(7682):639–43

Anding AL, Baehrecke EH. 2015. Autophagy in cell life and cell death. *Curr. Top. Dev. Biol.* 114:67–91

Bailey HH, Mulcahy RT, Tutsch KD, Arzoomanian RZ, Alberti D, et al. 1994. Phase I clinical trial of intravenous L-buthionine sulfoximine and melphalan: an attempt at modulation of glutathione. *J. Clin. Oncol.* 12(1):194–205

Bailey HH, Ripple G, Tutsch KD, Arzoomanian RZ, Alberti D, et al. 1997. Phase I study of continuous-infusion L-*S*,*R*-buthionine sulfoximine with intravenous melphalan. *J. Natl. Cancer Inst.* 89(23):1789–96

Banjac A, Perisic T, Sato H, Seiler A, Bannai S, et al. 2008. The cystine/cysteine cycle: a redox cycle regulating susceptibility versus resistance to cell death. *Oncogene* 27(11):1618–28

Basuli D, Tesfay L, Deng Z, Paul B, Yamamoto Y, et al. 2017. Iron addiction: a novel therapeutic target in ovarian cancer. *Oncogene* 36(29):4089–99

Brumatti G, Ma C, Lalaoui N, Nguyen N-Y, Navarro M, et al. 2016. The caspase-8 inhibitor emricasan combines with the SMAC mimetic birinapant to induce necroptosis and treat acute myeloid leukemia. *Sci. Transl. Med.* 8(339):339ra69

Brown CW, Amante JJ, Goel HL, Mercurio AM. 2017. The α6β4 integrin promotes resistance to ferroptosis. *J. Cell Biol.* 216(12):4287–97

Brown CW, Amante JJ, Mercurio AM. 2018. Cell clustering mediated by the adhesion protein PVRL4 is necessary for α6β4 integrin–promoted ferroptosis resistance in matrix-detached cells. *J. Biol Chem*. In press

Bump EA, Yu NY, Brown JM. 1982. Radiosensitization of hypoxic tumor cells by depletion of intracellular glutathione. *Science* 217(4559):544–45

Calvert P, Yao KS, Hamilton TC, O'Dwyer PJ. 1998. Clinical studies of reversal of drug resistance based on glutathione. *Chem. Biol. Interact.* 111–112:213–24

Cao JY, Dixon SJ. 2016. Mechanisms of ferroptosis. *Cell. Mol. Life Sci.* 73(11–12):2195–209

Chen D, Tavana O, Chu B, Erber L, Chen Y, et al. 2017. NRF2 is a major target of ARF in p53-independent tumor suppression. *Mol. Cell* 68(1):224–32

Chen W, Sun Z, Wang X-J, Jiang T, Huang Z, et al. 2009. Direct interaction between Nrf2 and $p21^{Cip1/WAF1}$ upregulates the Nrf2-mediated antioxidant response. *Mol. Cell* 34(6):663–73

Chintala S, Li W, Lamoreux ML, Ito S, Wakamatsu K, et al. 2005. *Slc7a11* gene controls production of pheomelanin pigment and proliferation of cultured cells. *PNAS* 102(31):10964–69

Chio IIC, Jafarnejad SM, Ponz-Sarvise M, Park Y, Rivera K, et al. 2016. NRF2 promotes tumor maintenance by modulating mRNA translation in pancreatic cancer. *Cell* 166(4):963–76

Conrad M, Kagan VE, Bayir H, Pagnussat GC, Head B, et al. 2018. Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev*. 32:602–19

Coriat R, Nicco C, Chéreau C, Mir O, Alexandre J, et al. 2012. Sorafenib-induced hepatocellular carcinoma cell death depends on reactive oxygen species production in vitro and in vivo. *Mol. Cancer Ther.* 11(10):2284–93

Cramer SL, Saha A, Liu J, Tadi S, Tiziani S, et al. 2017. Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. *Nat. Med.* 23(1):120–27

Delbridge ARD, Valente LJ, Strasser A. 2012. The role of the apoptotic machinery in tumor suppression. *Cold Spring Harb. Perspect. Biol.* 4(11):a008789

Demierre M-F, Higgins PDR, Gruber SB, Hawk E, Lippman SM. 2005. Statins and cancer prevention. *Nat. Rev. Cancer* 5(12):930–42

DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, et al. 2011. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475(7354):106–9

Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, et al. 2009. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 458(7239):780–83

Dixon SJ. 2017. Ferroptosis: bug or feature? *Immunol. Rev.* 277(1):150–57

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, et al. 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149(5):1060–72

Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, et al. 2014. Pharmacological inhibition of cystine–glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *eLife* 3:e02523

Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, et al. 2015. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem. Biol.* 10(7):1604–9

Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, et al. 2017. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13(1):91–98

Dolma S, Lessnick SL, Hahn WC, Stockwell BR. 2003. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell* 3(3):285–96

Evan GI, Vousden KH. 2001. Proliferation, cell cycle and apoptosis in cancer. *Nature* 411(6835):342–48

Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, et al. 2014. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat. Cell Biol.* 16(12):1180–91

Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. 2018. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ*. 25:486–541

Gao M, Jiang X. 2017. To eat or not to eat—the metabolic flavor of ferroptosis. *Curr. Opin. Cell Biol.* 51:58–64

Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. 2016. Ferroptosis is an autophagic cell death process. *Cell Res.* 26(9):1021–32

Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. 2015. Glutaminolysis and transferrin regulate ferroptosis. *Mol. Cell* 59(2):298–308

Gascón S, Murenu E, Masserdotti G, Ortega F, Russo GL, et al. 2016. Identification and successful negotiation of a metabolic checkpoint in direct neuronal reprogramming. *Cell Stem Cell* 18(3):396–409

Gaschler MM, Andia AA, Liu H, Csuka JM, Hurlocker B, et al. 2018. $FINO_2$ initiates ferroptosis through GPX4 inactivation and iron oxidation. *Nat. Chem. Biol.* 14:507–15

Gaschler MM, Stockwell BR. 2017. Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* 482(3):419–25

Giampazolias E, Zunino B, Dhayade S, Bock F, Cloix C, et al. 2017. Mitochondrial permeabilization engages NF-κB-dependent anti-tumour activity under caspase deficiency. *Nat. Cell Biol.* 19(9):1116–29

Gout PW, Buckley AR, Simms CR, Bruchovsky N. 2001. Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x_c^- cystine transporter: a new action for an old drug. *Leukemia* 15(10):1633–40

Green DR, Victor B. 2012. The pantheon of the fallen: Why are there so many forms of cell death? *Trends Cell Biol.* 22(11):555–56

Griffith OW, Meister A. 1979. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). *J. Biol. Chem.* 254(16):7558–60

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* 144(5):646–74

Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, et al. 2017. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 551(7679):247–50

Harris IS, Treloar AE, Inoue S, Sasaki M, Gorrini C, et al. 2015. Glutathione and thioredoxin antioxidant pathways synergize to drive cancer initiation and progression. *Cancer Cell* 27(2):211–22

Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, et al. 2016. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat. Med.* 22(3):262–69

Hou W, Xie Y, Song X, Sun X, Lotze MT, et al. 2016. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12(8):1425–28

Inde Z, Dixon SJ. 2018. The impact of non-genetic heterogeneity on cancer cell death. *Crit. Rev. Biochem. Mol. Biol.* 53(1):99–114

Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, et al. 2018. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* 172(3):409–22

Ishimoto T, Nagano O, Yae T, Tamada M, Motohara 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(3):387–400

Jaramillo MC, Zhang DD. 2013. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev.* 27(20):2179–91

Jennis M, Kung C-P, Basu S, Budina-Kolomets A, Leu JI-J, et al. 2016. An African-specific polymorphism in the *TP53* gene impairs p53 tumor suppressor function in a mouse model. *Genes Dev.* 30(8):918–30

Jiang L, Kon N, Li T, Wang S-J, Su T, et al. 2015. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520(7545):57–62

Johnstone RW, Ruefli AA, Lowe SW. 2002. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* 108(2):153–64

Kagan VE, Mao G, Qu F, Angeli JPF, Doll S, et al. 2017. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* 13(1):81–90

Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, et al. 2015. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526(7575):666–71

Kerins MJ, Ooi A. 2018. The roles of NRF2 in modulating cellular iron homeostasis. *Antioxid. Redox Signal.* 29:1756–73

Kim SE, Zhang L, Ma K, Riegman M, Chen F, et al. 2016. Ultrasmall nanoparticles induce ferroptosis in nutrient-deprived cancer cells and suppress tumour growth. *Nat. Nanotechnol.* 11(11):977–85

Koo G-B, Morgan MJ, Lee D-G, Kim W-J, Yoon J-H, et al. 2015. Methylation-dependent loss of RIP3 expression in cancer represses programmed necrosis in response to chemotherapeutics. *Cell Res.* 25(6):707–25

Korsmeyer SJ. 1999. *BCL-2* gene family and the regulation of programmed cell death. *Cancer Res.* 59(7 Suppl):1693s–700s

Kruiswijk F, Labuschagne CF, Vousden KH. 2015. p53 in survival, death and metabolic health: a lifeguard with a licence to kill. *Nat. Rev. Mol. Cell Biol.* 16(7):393–405

Lachaier E, Louandre C, Godin C, Saidak Z, Baert M, et al. 2014. Sorafenib induces ferroptosis in human cancer cell lines originating from different solid tumors. *Anticancer Res.* 34(11):6417–22

Larraufie M-H, Yang WS, Jiang E, Thomas AG, Slusher BS, Stockwell BR. 2015. Incorporation of metabolically stable ketones into a small molecule probe to increase potency and water solubility. *Bioorg. Med. Chem. Lett.* 25(21):4787–92

Li T, Kon N, Jiang L, Tan M, Ludwig T, et al. 2012. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. *Cell* 149(6):1269–83

Lien EC, Lyssiotis CA, Juvekar A, Hu H, Asara JM, et al. 2016. Glutathione biosynthesis is a metabolic vulnerability in PI(3)K/Akt-driven breast cancer. *Nat. Cell Biol.* 18(5):572–78

Linkermann A, Skouta R, Himmerkus N, Mulay SR, Dewitz C, et al. 2014. Synchronized renal tubular cell death involves ferroptosis. *PNAS* 111(47):16836–41

Liu DS, Duong CP, Haupt S, Montgomery KG, House CM, et al. 2017. Inhibiting the system x_C^-/glutathione axis selectively targets cancers with mutant-p53 accumulation. *Nat Commun.* 8:14844

Louandre C, Ezzoukhry Z, Godin C, Barbare J-C, Mazière J-C, et al. 2013. Iron-dependent cell death of hepatocellular carcinoma cells exposed to sorafenib. *Int. J. Cancer* 133(7):1732–42

Maddocks ODK, Berkers CR, Mason SM, Zheng L, Blyth K, et al. 2013. Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* 493(7433):542–46

Magtanong L, Ko PJ, Dixon SJ. 2016. Emerging roles for lipids in non-apoptotic cell death. *Cell Death Differ.* 23:1099–109

Mai TT, Hamaï A, Hienzsch A, Cañeque T, Müller S, et al. 2017. Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat. Chem.* 9(10):1025–33

Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. 2014. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 509(7498):105–9

Moosmann B, Behl C. 2004. Selenoprotein synthesis and side-effects of statins. *Lancet* 363(9412):892–94

Ni Chonghaile T, Sarosiek KA, Vo T-T, Ryan JA, Tammareddi A, et al. 2011. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. *Science* 334(6059):1129–33

Poursaitidis I, Wang X, Crighton T, Labuschagne C, Mason D, et al. 2017. Oncogene-selective sensitivity to synchronous cell death following modulation of the amino acid nutrient cystine. *Cell Rep.* 18(11):2547–56

Qi X-F, Kim D-H, Yoon Y-S, Kim S-K, Cai D-Q, et al. 2010. Involvement of oxidative stress in simvastatin-induced apoptosis of murine CT26 colon carcinoma cells. *Toxicol. Lett.* 199(3):277–87

Robe PA, Martin DH, Nguyen-Khac MT, Artesi M, Deprez M, et al. 2009. Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults. *BMC Cancer* 9(1):372

Roh J-L, Kim EH, Jang H, Shin D. 2017. Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. *Redox Biol.* 11:254–62

Romero R, Sayin VI, Davidson SM, Bauer MR, Singh SX, et al. 2017. *Keap1* loss promotes *Kras*-driven lung cancer and results in dependence on glutaminolysis. *Nat. Med.* 23(11):1362–68

Sarosiek KA, Fraser C, Muthalagu N, Bhola PD, Chang W, et al. 2017. Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. *Cancer Cell* 31(1):142–56

Sarosiek KA, Ni Chonghaile T, Letai A. 2013. Mitochondria: gatekeepers of response to chemotherapy. *Trends Cell Biol.* 23(12):612–19

Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, et al. 2005. Redox imbalance in cystine/glutamate transporter-deficient mice. *J. Biol. Chem.* 280(45):37423–29

Sato H, Tamba M, Ishii T, Bannai S. 1999. Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *J. Biol. Chem.* 274(17):11455–58

Schoenfeld JD, Sibenaller ZA, Mapuskar KA, Wagner BA, Cramer-Morales KL, et al. 2017. $O_2^{\cdot-}$ and H_2O_2-mediated disruption of Fe metabolism causes the differential susceptibility of NSCLC and GBM cancer cells to pharmacological ascorbate. *Cancer Cell* 31(4):487–88

Schonberg DL, Miller TE, Wu Q, Flavahan WA, Das NK, et al. 2015. Preferential iron trafficking characterizes glioblastoma stem-like cells. *Cancer Cell* 28(4):441–55

Schott C, Graab U, Cuvelier N, Hahn H, Fulda S. 2015. Oncogenic RAS mutants confer resistance of RMS13 rhabdomyosarcoma cells to oxidative stress-induced ferroptotic cell death. *Front. Oncol.* 5:131

Seiler A, Schneider M, Förster H, Roth S, Wirth EK, et al. 2008. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* 8(3):237–48

Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, et al. 2010. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 141(1):69–80

Shimada K, Hayano M, Pagano NC, Stockwell BR. 2016a. Cell-line selectivity improves the predictive power of pharmacogenomic analyses and helps identify NADPH as biomarker for ferroptosis sensitivity. *Cell Chem. Biol.* 23(2):225–35

Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, et al. 2016b. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat. Chem. Biol.* 12(7):497–503

Shitara K, Doi T, Nagano O, Imamura CK, Ozeki T, et al. 2017. Dose-escalation study for the targeting of $CD44v^+$ cancer stem cells by sulfasalazine in patients with advanced gastric cancer (EPOC1205). *Gastric Cancer* 20(2):341–49

Singh A, Venkannagari S, Oh KH, Zhang Y-Q, Rohde JM, et al. 2016. Small molecule inhibitor of NRF2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors. *ACS Chem. Biol.* 11(11):3214–25

Skouta R, Dixon SJ, Wang J, Dunn DE, Orman M, et al. 2014. Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *J. Am. Chem. Soc.* 136(12):4551–56

Somfai-Relle S, Suzukake K, Vistica BP, Vistica DT. 1984. Reduction in cellular glutathione by buthionine sulfoximine and sensitization of murine tumor cells resistant to L-phenylalanine mustard. *Biochem. Pharmacol.* 33(3):485–90

Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, et al. 2017. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171(2):273–85

Sun X, Ou Z, Chen R, Niu X, Chen D, et al. 2016. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology* 63(1):173–84

Tarangelo A, Magtanong L, Bieging-Rolett KT, Li Y, Ye J, et al. 2018. p53 suppresses metabolic stress-induced ferroptosis in cancer cells. *Cell Rep.* 22(3):569–75

Timmerman LA, Holton T, Yuneva M, Louie RJ, Padró M, et al. 2013. Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. *Cancer Cell.* 24(4):450–65

Torii S, Shintoku R, Kubota C, Yaegashi M, Torii R, et al. 2016. An essential role for functional lysosomes in ferroptosis of cancer cells. *Biochem. J.* 473(6):769–77

Torti SV, Torti FM. 2013. Iron and cancer: more ore to be mined. *Nat. Rev. Cancer* 13(5):342–55

Tsoi J, Robert L, Paraiso K, Galvan C, Sheu KM, et al. 2018. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. *Cancer Cell* 33(5):890–904

Ursini F, Maiorino M, Gregolin C. 1985. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim. Biophys. Acta* 839(1):62–70

Villablanca JG, Volchenboum SL, Cho H, Kang MH, Cohn SL, et al. 2016. A phase I new approaches to neuroblastoma therapy study of buthionine sulfoximine and melphalan with autologous stem cells for recurrent/refractory high-risk neuroblastoma. *Pediatr. Blood Cancer* 63(8):1349–56

Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, et al. 2017. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* 547(7664):453–57

Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, et al. 2003. *Keap1*-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat. Genet.* 35(3):238–45

Wang GX, Tu H-C, Dong Y, Skanderup AJ, Wang Y, et al. 2017. ΔNp63 inhibits oxidative stress-induced cell death, including ferroptosis, and cooperates with the BCL-2 family to promote clonogenic survival. *Cell Rep.* 21(10):2926–39

Wang S-J, Li D, Ou Y, Jiang L, Chen Y, et al. 2016. Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. *Cell Rep*. 17(2):366–73

Wang Y, Gao W, Shi X, Ding J, Liu W, et al. 2017. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature* 547(7661):99–103

Warner GJ, Berry MJ, Moustafa ME, Carlson BA, Hatfield DL, Faust JR. 2000. Inhibition of selenoprotein synthesis by selenocysteine tRNA$^{[Ser]Sec}$ lacking isopentenyladenosine. *J. Biol. Chem.* 275(36):28110–19

Weiwer M, Bittker J, Lewis TA, Shimada K, Yang WS, et al. 2012. Development of small-molecule probes that selectively kill cells induced to express mutant RAS. *Bioorg. Med. Chem. Lett.* 22(4):1822–26

Wenzel SE, Tyurina YY, Zhao J, St. Croix CM, Dar HH, et al. 2017. PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell* 171(3):628–41.e26

Woo JH, Shimoni Y, Yang WS, Subramaniam P, Iyer A, et al. 2015. Elucidating compound mechanism of action by network perturbation analysis. *Cell* 162(2):441–51

Xie Y, Zhu S, Song X, Sun X, Fan Y, et al. 2017. The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. *Cell Rep*. 20(7):1692–704

Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, et al. 2007. RAS–RAF–MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature* 447(7146):864–68

Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. 2016. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *PNAS* 113(34):E4966–75

Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, et al. 2014. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156(1–2):317–31

Yang WS, Stockwell BR. 2008. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem. Biol.* 15(3):234–45

Yang WS, Stockwell BR. 2016. Ferroptosis: death by lipid peroxidation. *Trends Cell Biol*. 26(3):165–76

Yuan H, Li X, Zhang X, Kang R, Tang D. 2016a. CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. *Biochem. Biophys. Res. Commun.* 478(2):838–44

Yuan H, Li X, Zhang X, Kang R, Tang D. 2016b. Identification of ACSL4 as a biomarker and contributor of ferroptosis. *Biochem. Biophys. Res. Commun.* 478(3):1338–43

Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, et al. 2015. Vitamin C selectively kills *KRAS* and *BRAF* mutant colorectal cancer cells by targeting GAPDH. *Science* 350(6266):1391–96

Zilka O, Shah R, Li B, Friedmann Angeli JP, Griesser M, et al. 2017. On the mechanism of cytoprotection by ferrostatin-1 and liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. *ACS Cent. Sci.* 3(3):232–43