Received: 23 August 2021 | Revised: 11 September 2021 | Accepted: 30 September 2021

DOI: 10.1002/cac2.12231

CANCER COMMUNICATIONS Open Access

EDITORIAL

Phospholipase iPLA2β acts as a guardian against ferroptosis

Ferroptosis, a form of iron-dependent regulated cell death caused by excessive accumulation of lipid hydroperoxides, has been associated with various pathological conditions and diseases [1]. Excessive ferroptosis has been causally associated with acute kidney injury, cardiovascular, neurodegenerative and hepatic diseases, whereas impaired ferroptosis in premalignant cells has been shown to contribute to tumor development [2,3]. To escape from ferroptotic cell death, cells have been equipped with several antioxidant defense systems against lipid peroxidation (Figure 1). Glutathione peroxidase 4 (GPX4) suppresses ferroptosis by converting lipid hydroperoxides into non-toxic lipid alcohols at the expense of its cofactor glutathione (GSH) [4]. Ferroptosis suppressor protein-1 (FSP1, also known as AIFM2), a NAD(P)H-dependent oxidoreductase located on the plasma membrane, catalyzes the reduction of ubiquinone to ubiquinol, a radical trapping antioxidant that suppresses ferroptosis independent of the GSH-GPX4 axis [5,6]. In addition, dihydroorotate dehydrogenase (DHODH), an enzyme involved in the *de novo* pyrimidine biosynthesis pathway, inhibits ferroptosis by reducing ubiquinone to ubiquinol in the inner mitochondrial membrane [7].

Most lipid peroxidation occurs in polyunsaturated fatty acid-containing phospholipids (PUFA-PLs; PUFAs are fatty acids that harbor more than one double bond). Due to the chemical features of double bonds in PUFAs, PUFA-PLs are exquisitely vulnerable to peroxidation in cellular environments rich in iron and oxygen. One major oxidized PUFA-PL species that is believed to trigger ferroptosis is 15-hydroperoxy-arachidonoyl-phosphatidylethanolamine (15-HpETE-PE) [8]. However, how cells neutralize 15-HpETE-PE (and other oxidized PUFA-PLs) and escape from ferroptotic cell death remains incompletely understood.

The phospholipase A_2 (PLA_2) gene family encodes protein enzymes that specifically hydrolyze the sn-2 ester bond on PLs, yielding free fatty acids and lysophosphatidic acids. Calcium-independent phospholipase A2β (iPLA2β), a member of the PLA_2 family, is a cytosolic protein that is catalytically active in the absence of calcium and can be stimulated by ATP. In response to stress or injury, iPLA2β preferentially releases free fatty acids (particularly PUFAs, such as arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid) from the sn-2 position of PLs. While iPLA2β was initially implicated as a housekeeping enzyme in membrane remodeling, recent studies have suggested an engaged role of iPLA2β in a variety of cellular processes, including calcium homeostasis, apoptosis, and inflammation, and its dysregulation has been genetically linked to diverse human diseases, such as Parkinson's disease (PD), male infertility, cardiovascular abnormalities, and cancer [9]. However, the underlying mechanisms by which iPLA2β dysregulation is linked to these human diseases or pathological conditions still remain largely unknown. Two recent studies revealed that iPLA2β preferentially hydrolyzes peroxidized PLs, such as 15-HpETE-PE, and acts as a GPX4-independent repressor of p53-driven ferroptosis [10,11]. These findings further suggest that iPLA2β is a promising therapeutic target for cancer therapy and that its mutation may be relevant to PD.

Sun et al. [10] examined the specific hydrolytic activity of iPLA2β, revealing that both 1-stearoyl (SA)-2-ETE-PE and 1-SA-2-15-HpETE-PE (one specific 15-HpETE-PE) were highly hydrolyzed by iPLA2β. Further studies from both experimental analyses and computational modeling suggested that 1-SA-2-15-HpETE-PE is likely the preferred substrate for iPLA2β. R747W is a loss-of-function mutant in *iPLA2β* that is associated with infantile neuroaxonal dystrophy and adult-onset dystonia-parkinsonism. Notably, the R747W mutant was shown to exhibit lower hydrolytic activity toward 1-SA-2-15-HpETE-PE than did the wild-type iPLA2β protein. Likewise, the phospholipase

Abbreviations: GPX4, Glutathione peroxidase 4; FSP1, Ferroptosis suppressor protein-1; DHODH, Dihydroorotate dehydrogenase; PUFA-PLs, polyunsaturated-fatty-acid–containing phospholipids; 15-HpETE-PE, 15-hydroperoxy-arachidonoyl-phosphatidylethanolamine; PLA2, phospholipase A2; iPLA2β, Calcium-independent phospholipase A2β; PD, Parkinson's disease; 4-HNE, 4-hydroxynonenal

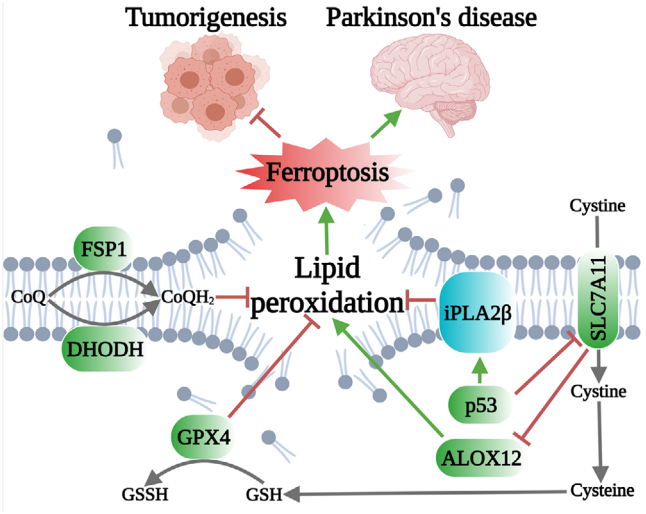

FIGURE 1 Several cellular defense systems against lipid peroxidation and ferroptosis, and the relevance of ferroptosis to tumorigenesis and Parkinson's disease. GPX4 detoxifies lipid hydroperoxides and represses ferroptosis by converting reduced GSH into GSSG. FSP1 and DHODH catalyze the reduction of CoQ to $CoQH_2$ on the plasma membrane and inner mitochondrial membrane, respectively; $CoQH_2$ acts as a radical trapping antioxidant to suppress lipid peroxidation and ferroptosis. p53 appears to have two opposing functions in ferroptosis. p53 promotes ferroptosis at least partly by suppressing the expression of SLC7A11, which inhibits ferroptosis through both GPX4-dependent (via GSH) and -independent (via ALOX12) pathways. On the other hand, p53 upregulates the expression of iPLA2*β*, which suppresses ferroptosis. iPLA2*β* inactivation suppresses tumorigenesis and promotes Parkinson's disease at least partly through promoting ferroptosis. Abbreviations: GPX4, glutathione peroxidase 4; GSH, glutathione; GSSG, oxidized glutathione; FSP1, ferroptosis suppressor protein-1; DHODH, dihydroorotate dehydrogenase; CoQ, ubiquinone; $CoQH_2$, ubiquinol; SLC7A11, solute carrier family 7 member 11; ALOX12, arachidonate 12-lipoxygenase; iPLA2*β*, calcium-independent phospholipase A2*β*.

activity of iPLA2*β* toward 1-SA-2-15-HpETE-PE was dramatically lower in cells harboring PD-associated mutation (fPD^{R747W}) than in wild-type counterparts. Mechanistically, it was proposed that R747W iPLA2*β* mutant protein has less accessible catalytic site and weaker membrane association as compared with the wild-type iPLA2*β* protein, which likely explains its lower hydrolytic activity toward its substrate 1-SA-2-15-HpETE-PE. Given the important role of 15-HpETE-PE in driving ferroptosis, these studies further suggest that iPLA2*β* might suppress ferroptosis by hydrolyzing 15-HpETE-PE. In support of this hypothesis, Sun et al. [10] showed that fPD^{R747W} cells contained more oxidized PEs and exhibited more sensitivities to GPX4 inhibitor RSL3-induced ferroptosis than did wild-type cells.

In another study, Chen et al. [11] uncovered a role of iPLA2*β* in ferroptosis governance through their efforts to understand tumor suppressor p53's function in ferroptosis. Combined treatment of *tert*-butyl hydroperoxide (a ferroptosis inducer) and Nutlin (a p53 activator) was found to induce high levels of lipid peroxidation and to result in potent p53-dependent ferroptotic cell death; notably, p53-driven ferroptosis appeared to be independent of GPX4. This prompted the authors to identify additional downstream targets of p53 to control ferroptosis. Through mining RNA-sequencing data, Chen et al. [11] revealed that *iPLA2β* is a direct transcriptional target of p53; both pharmacological and genetic activation of p53 significantly upregulated *iPLA2β* mRNA levels, whereas p53 deficiency dampened Nutlin-induced *iPLA2β* expression. They further showed that *iPLA2β* depletion promoted ferroptosis in *p53* wild-type cells but not in *p53*-null counterparts. In addition, overexpression of iPLA2*β*, but not its enzymatic inactive mutant, was found to reduce the levels of oxidized PEs and to inhibit p53-driven ferroptosis. Collectively, these data suggest that iPLA2*β* functions to suppress p53-induced ferroptosis likely through hydrolyzing oxidized PUFA-PLs. It should be noted that although p53 promotes *iPLA2β* expression, p53 and iPLA2*β* have opposite functions in regulating ferroptosis: p53 promotes whereas iPLA2*β* suppresses ferroptosis. Therefore, iPLA2*β* is unlikely the p53 transcriptional target that mediates p53's function in promoting ferroptosis; rather, it seems that iPLA2*β* as a p53's target serves to buffer p53's ability to induce ferroptosis (Figure 1).

The above two studies also examined the disease relevance of iPLA2*β*'s function in ferroptosis governance. Chen et al. [11] showed that iPLA2*β* deficiency suppressed xenograft tumor growth and increased ferroptosis marker expression in *p53* wild-type but not in *p53*-null tumors, suggesting that iPLA2*β* could promote tumor growth in a p53-dependent manner and possibly through suppressing ferroptosis in vivo. It should be noted that while *iPLA2β* is a transcriptional target of p53, the dependency of iPLA2*β*-mediated regulation of ferroptosis and tumor growth on p53 suggests that somehow p53 can also operate downstream of iPLA2*β*, although the underlying mechanism remains unclear. Sun et al. [10] studied iPLA2*β* in the context of PD. iPLA2*β* is encoded by the *PNPLA9* gene. Since mouse *Pnpla9* R748W mutant corresponds to human *PNPLA9* R747W mutant (which is associated with PD), they developed *Pnpla9* $^{R748W/R748W}$ mouse models and found that *Pnpla9* $^{R748W/R748W}$ mice indeed developed behavioral defects related to PD pathogenesis, with increased levels of 15-HpETE-PE and 4-hydroxynonenal (4-HNE, a lipid peroxidation marker) in midbrains of these mice compared with those in wild-type mice. In addition, the levels of tyrosine hydroxylase, a biomarker for

dopaminergic neurons in the central nervous system and its product dopamine were found to be significantly lower in the midbrain of *Pnpla9*$^{R748W/R748W}$ mutant mice than in control mice. These analyses suggest a causal effect of ferroptosis in mediating iPLA2*β* mutation's function in PD pathogenesis.

Together, these two intriguing studies [10,11] reveal that iPLA2*β* acts as a guardian against ferroptosis by hydrolyzing and thereby eliminating pro-ferroptotic oxidized PUFA-PLs, and further suggest that iPLA2*β* deficiency or its loss-of-function mutation can suppress tumor growth or promote PD pathogenesis, potentially by inducing aberrant ferroptosis (Figure 1). These studies also raised several outstanding questions. Since the PLA2 gene family encodes multiple phospholipases, whether other PLA2 members are also involved in ferroptosis governance remains to be investigated. iPLA2*β* normally localizes in the cytosol, but upon stimulation it can mobilize to organelles such as the endoplasmic reticulum, mitochondria, and nucleus. For example, iPLA2*β* has been implicated in repairing mitochondrial phospholipids [12]. In light of recent findings suggesting separate ferroptosis defense systems on the plasma membrane and in the mitochondria in cells [7,13], whether iPLA2*β* participates in ferroptosis defense in different subcellular compartments under diverse cellular conditions remains a fascinating area for future studies. In addition, although these two studies have causally linked ferroptosis to iPLA2*β*'s functions in tumor biology and PD pathogenesis, the extent to which ferroptosis contributes to tumor suppression or PD-associated phenotypes caused by *iPLA2β* deletion or mutation remains unclear. Considering the multifaceted roles of iPLA2*β* in cellular signaling, other biological functions of iPLA2*β* might also be involved. Rescue analyses by ferroptosis inhibitors such as liproxstatin-1 or ferrostatin-1 will help address this important question. Further, as a key transcription factor involved in ferroptosis regulation, p53's activity is tightly regulated by post-translational modifications such as phosphorylation and acetylation. For example, the suppressor of cytokine signaling 1 (SOCS1) reduces the expression of SLC7A11 through promoting p53 serine-15 phosphorylation and thereby sensitizes cells to ferroptosis [14]. In addition, p53 acetylation at lysine K98 is essential for its ability to inhibit SLC7A11 expression and to promote ferroptosis in mouse xenograft models [15]. Whether these post-translational modifications of p53 are also involved in regulating *iPLA2β* expression remains to be tested. Likewise, it will be interesting to identify other transcription factors that regulate *iPLA2β* expression and ferroptosis in future investigations. Finally, from a therapeutic perspective, the study by Chen et al. [11] suggests iPLA2*β* inhibitors as potential therapeutic strategies in treating *p53* wild-type tumors. However, the study by Sun et al. [10] would argue that such therapeutic interventions might cause other complications, including promoting PDs. It remains to be established whether there exist appropriate therapeutic windows for iPLA2*β* inhibitors to selectively kill tumors without inducing obvious toxicities in the brain and other organs. Future investigations will be directed to understanding these important questions.

DECLARATIONS

AUTHORS' CONTRIBUTIONS

CM drafted the manuscript with additional support from GL for checking relevant literature. BG provided critical revision of the manuscript with additional manuscript editing support from LZ. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

Not applicable.

COMPETING INTERESTS

BG is an inventor of patent applications involving targeting ferroptosis in cancer therapy. Other authors have no conflicts of interest to declare.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

Research in the authors' lab has been supported by The University of Texas MD Anderson Cancer Center, National Institutes of Health grants R01CA181196, R01CA244144, and R01CA247992 (to BG) and by Cancer Center Support (Core) Grant P30 CA016672 from the National Cancer Institute (to The University of Texas MD Anderson Cancer Center).

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