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Perspective

How do different lipid peroxidation mechanisms contribute to ferroptosis?

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SUMMARY

Lipid peroxidation is the driver of ferroptotic cell death. However, nonconjugated and conjugated polyunsaturated fatty acids potentiate ferroptosis differently, while some isoprenoid-derived lipids inhibit ferroptosis despite being highly oxidizable. In this perspective, we propose that different oxidation mechanisms and products contribute to the discrepancies in the lipids' potency in modulating ferroptosis. We first discuss the relative reactivities of various lipids toward two rate-determining free radical propagating mechanisms, hydrogen atom transfer (HAT) and peroxyl radical addition (PRA), and the resulting differential product profiles. We then discuss the role and regulation of lipid peroxidation in ferroptosis and the potential contributions of different oxidation products, such as truncated lipids and lipid electrophiles, from HAT and PRA mechanisms to the execution of ferroptosis. Lastly, we offer our perspective on the remaining questions to fully understand the process from lipid peroxidation to ferroptosis.

INTRODUCTION

Lipids are among the primary targets of attack by free radical species formed under oxidative stress conditions, leading to free radical chain reactions with molecular oxygen, termed lipid peroxidation.[1] Lipid peroxidation has been closely associated with various degenerative diseases.[2–4] More recently, lipid peroxidation of membrane-incorporated polyunsaturated fatty acids (PUFAs) was found to drive ferroptosis, a regulated form of cell death that is iron dependent and distinct from apoptosis, necrosis, and autophagy.[5–10] Glutathione peroxidase 4 (GPX4) plays a central role in the inhibition of ferroptosis, as it is the only GPX isoform that can reduce lipid peroxides inside the lipid membranes to alcohols using glutathione (GSH) as a cofactor.[6] Restricting access to the precursor of GSH, cysteine, by inhibiting the cystine/glutamate antiporter x_c^- or by direct inhibition of GPX4 induces ferroptosis.[5,6] Back in 2003, the Stockwell Lab identified a small molecule, erastin, that induces a form of nonapoptotic cell death,[11] which was later found to be dependent on the formation of oxidative species.[12] In 2008, the same group discovered that another small molecule, RSL3, induces cell death in a similar manner and that the cell death is iron dependent.[13] At about the same time, the Conrad Lab discovered that GPX4 inactivation led to increased lipid peroxidation and, subsequently, an unrecognized mode of cell death.[14] In 2012, the Stockwell Lab officially coined the term "ferroptosis" to describe the erastin- and RSL3-induced cell death.[5] They further established that erastin inhibits x_c^-, while RSL3 directly inhibits GPX4 through a covalent modification.[5,6]

Since the discovery of ferroptosis, several biologically important lipids have been reported to modulate ferroptosis sensitivity. Specifically, exogenous peroxidation-reactive nonconjugated PUFAs, including linoleic acid (LA) and arachidonic acid

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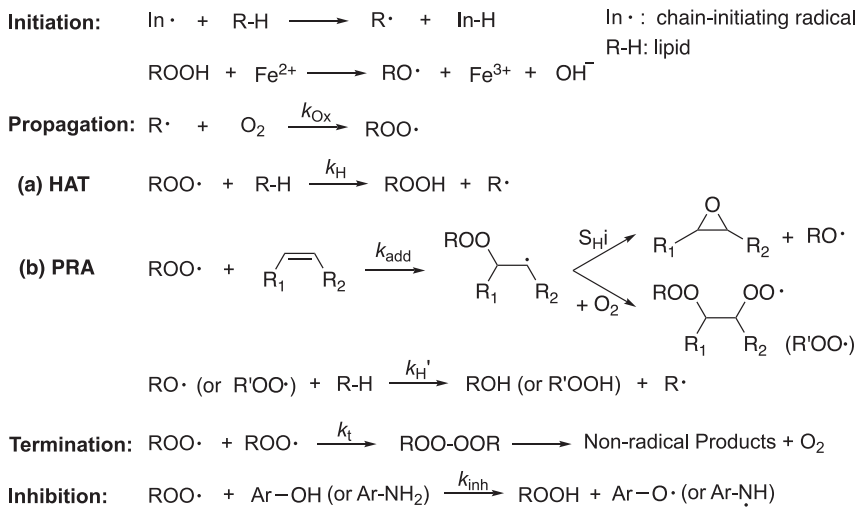

Figure 1. Important steps of free radical chain oxidation reactions

HAT, hydrogen atom transfer; PRA, peroxyl radical addition; S_Hi, intramolecular homolytic substitution; k_{ox}, oxygen addition rate constant; k_H, HAT rate constant; k_{add}, PRA rate constant; k_t, termination rate constant; k_{inh}, inhibition rate constant.

(AA),[1] were shown to sensitize cancer cells to ferroptosis.[7] In contrast, treatment with monounsaturated fatty acid oleic acid (OA), which has low reactivity to lipid peroxidation,[1] leads to resistance to ferroptosis.[15] In recent work by us and others, it was found that conjugated PUFAs are much more potent in inducing or potentiating ferroptosis than their nonconjugated counterparts.[16,17] However, not all lipid peroxidation leads to ferroptosis. For example, it was reported that a highly oxidizable sterol, 7-dehydrocholesterol (7-DHC),[18,19] can rescue ferroptosis.[16,20,21] These observations merit an in-depth investigation on the relationship between lipid peroxidation mechanisms and ferroptosis. Here, we propose that the different oxidation mechanisms and products may contribute to the discrepancies in the lipids' potency in modulating ferroptosis.

Free radical chain oxidation, or autoxidation, of lipids proceeds via a sequence of three steps: initiation, propagation, and termination (Figure 1). The rate-determining step in this sequence is the propagation step, where the lipid peroxyl radical can typically undergo two types of reactions: hydrogen atom transfer (HAT; k_H [HAT rate constant]) from a hydrogen atom donor to the peroxyl radical or peroxyl radical addition (PRA; k_{add} [PRA rate constant]) to a "C=C" double-bond system.[1,19] Thus, the propagation rate constant (k_p) of a given lipid would be the sum of k_H and k_{add}. When a radical-trapping antioxidant, such as α-tocopherol (the major form of vitamin E) or aryl amines, is present, the chain reaction is inhibited because k_{inh} (inhibition rate constant) usually is much larger than the sum of k_H and k_{add}, and the aryloxyl/arylaminyl radical formed is relatively stable. The long half-life of the aryloxyl or arylaminyl radical allows them to trap another radical (themselves or another lipid-derived radical) through radical-radical termination.[22]

In a biological environment, iron plays an important role in initiating new chain reactions by reacting with hydroperoxides, generating alkoxyl radicals, which can continue the chain reactions (Figure 1). The k_p values tend to be smaller in lipid membrane than in solution due to the decreased diffusion rates in the membrane.[18] Thus, lipid composition could affect the k_p values by modifying the membrane fluidity. For example, cholesterol exerts antiferroptotic properties, as it tends to decrease the

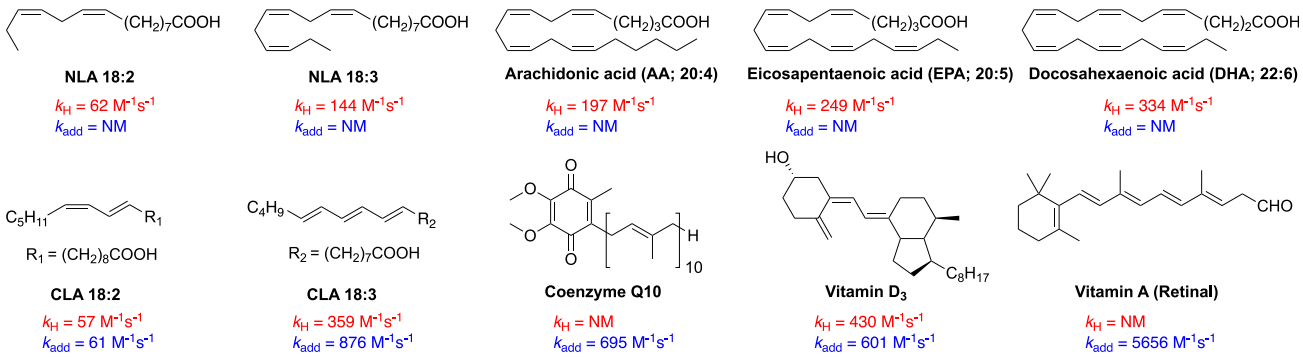

Figure 2. Structures and propagation rate constants of biologically important lipids

k_H, HAT rate constant; k_{add}, PRA rate constant; NM, not measurable.

membrane fluidity,[23] thus decreasing the lipid peroxidation k_p values in the membrane. On the other hand, PUFAs tend to increase the fluidity of the membrane,[24] further accelerating lipid peroxidation.

In this perspective, we will first discuss the relative reactivities of various lipids toward HAT and PRA mechanisms as measured using peroxyl radical clocks. We will then discuss the differential product profiles formed from HAT and PRA mechanisms with a focus on the mechanisms of the formation of truncated lipids and lipid electrophiles. Subsequently, we will discuss the role and regulation of lipid peroxidation in ferroptosis and the potential contributions of different oxidation products from HAT and PRA mechanisms to the execution of ferroptosis. Lastly, we will offer our perspective on the remaining questions to fully understand the process from lipid peroxidation to ferroptosis.

REACTIVITIES OF VARIOUS LIPIDS TOWARD HAT AND PRA MECHANISMS

Endogenous PUFAs contain nonconjugated double bonds separated by bis-allylic methylene groups, which are prone to HAT reactions due to the reactive bis-allylic C–H bonds. In fact, k_p values of nonconjugated PUFAs are proportional to the number of bis-allylic methylene groups as measured by a radical clock based on the peroxidation of linoleate.[1] When the bis-allylic C–H bonds are replaced with C–D bonds, the k_H (k_D for the removal of the deuterium atom) values decrease significantly due to a large kinetic isotope effect (k_H/k_D = 9.3), which has been shown to confer protection against ferroptosis and other oxidative damage.[7,25] The rate constants for the PRA reactions of lipids had not been measured until our recent report on an improved radical clock.[19] Although k_{add} values of some hydrocarbons, such as 1,3-cyclohexadiene and styrene, have been measured previously using the rotating-sector method, the chain-carrying radicals in these reactions are different substrate-derived peroxyl radicals.[26,27] On the other hand, the rate constants measured using the radical clock are all calibrated to the same scale using the reactions of linoleate peroxyl radicals and thus are more comparable.[19] In this work, we confirmed that nonconjugated PUFAs underwent lipid peroxidation predominantly via the HAT mechanism and reported, for the first time, the k_H and/or the k_{add} for several important lipids with conjugated double bonds, including conjugated PUFAs, vitamins A and D_3, and the oxidized form of coenzyme Q10. We found that coenzyme Q10, vitamin D_3, and vitamin A (in the form of retinal) were all highly reactive toward free radical oxidation with k_p (k_H + k_{add}) values of 695, 1,031, and 5,656 $M^{-1}s^{-1}$, respectively (Figure 2), and have a major or predominant contribution from the

PRA mechanism, which is clearly due to the highly conjugated double bonds. Due to the presence of a tertiary allylic H atom, vitamin D_3 also has a significant contribution from the HAT mechanism.

Significantly, we discovered that conjugated PUFAs display much higher reactivities toward free radical oxidation, with PRA being the major oxidation mechanism, than their nonconjugated counterparts. Specifically, conjugated linoleic acid (CLA 18:2) is twice as reactive as nonconjugated linoleic acid (NLA 18:2) (118 vs. 62 $M^{-1}s^{-1}$), while CLA 18:3 is over 8 times as reactive as NLA 18:3 (1,235 vs. 144 $M^{-1}s^{-1}$) (Figure 2). The k_{add} and k_H rate constants of CLA 18:2, CLA 18:3, vitamin D_3, and vitamin A suggest that the more conjugated the system is, the larger the increase in both k_{add} and k_H, with k_{add} becoming predominant with three or more conjugated double bonds.

DIFFERENTIAL PRODUCT PROFILES FORMED FROM HAT AND PRA MECHANISMS

HAT reactions at the bis-allylic methylene groups in nonconjugated PUFAs result in pentadienyl radicals. Diffusion-controlled addition of molecular oxygen to such radicals leads to peroxyl radicals, producing the primary products, hydroperoxides, after another HAT reaction. The peroxyl radical intermediates can undergo a variety of rearrangement reactions, such as 5-*exo* cyclization to a double bond and subsequent transformations, PRA to a double-bond system, or radical-radical termination to give an alcohol and a ketone (Figure 3A). These reactions of PUFAs and sterols have been reviewed in detail previously.[1,28] On the other hand, PRA reactions tend to lead to the formation of epoxides after intramolecular homolytic substitution (S_Hi) or the addition of another molecular oxygen, forming peroxide dimers or oligomers if additional PRA reactions occur (Figures 3C and 3D).

Primary oxidation products can undergo secondary C–C cleavage reactions, leading to the formation of reactive aldehydes with a shortened carbon chain, i.e., truncated lipids. Different mechanisms of C–C cleavage have been well documented in the studies of the formation of 4-hydroxynonenal (HNE), arguably the most studied lipid electrophile.[29–31] The first mechanism is through the rearrangement of hydroperoxide via the Hock cleavage under the catalysis of protic or Lewis acids (Figure 3B).[30] However, a strong protic acid, such as hydrochloric acid or sulfonic acid, is needed for the reaction to occur under the physiological temperature of 37°C. While the strong acid environment may not be relevant in the human body except the stomach, Lewis acids, such as free ferric or ferrous ions, are elevated during ferroptosis and thus could promote such C–C cleavage.

The second mechanism involves PRA to the conjugated double bonds of primary hydroperoxide products of PUFA peroxidation, such as hydroperoxyoctadecadienoates (HPODEs) and hydroperoxyeicosatetraenoates (HPETEs) from the oxidation of linoleate and arachidonate, respectively (Figure 3C). This addition results in either an allylic epoxide after S_Hi or a triperoxide after the addition of another oxygen molecule and HAT. Decomposition of the vicinal peroxides then leads to C–C cleavage and the formation of two aldehydes. Because PRA can occur at the carbon close or distal to the existing hydroperoxyl group, two sets of aldehydes are expected, with half being α,β-unsaturated aldehydes. This mechanism is supported by the formation of the increased amount of HNE from 1-palmitoyl-2-linoleoylphosphatidylcholine [PLPC (16:0/18:2)], to 1,2-dilinoleoyl-sn-glycero-3phosphocholine [DLPC (18:2/18:2)], to cardiolipin with four linoleates (CL 18:2/18:2/18:2/18:2), in that order.[31] For the same total linoleate concentration, DLPC generated approximately

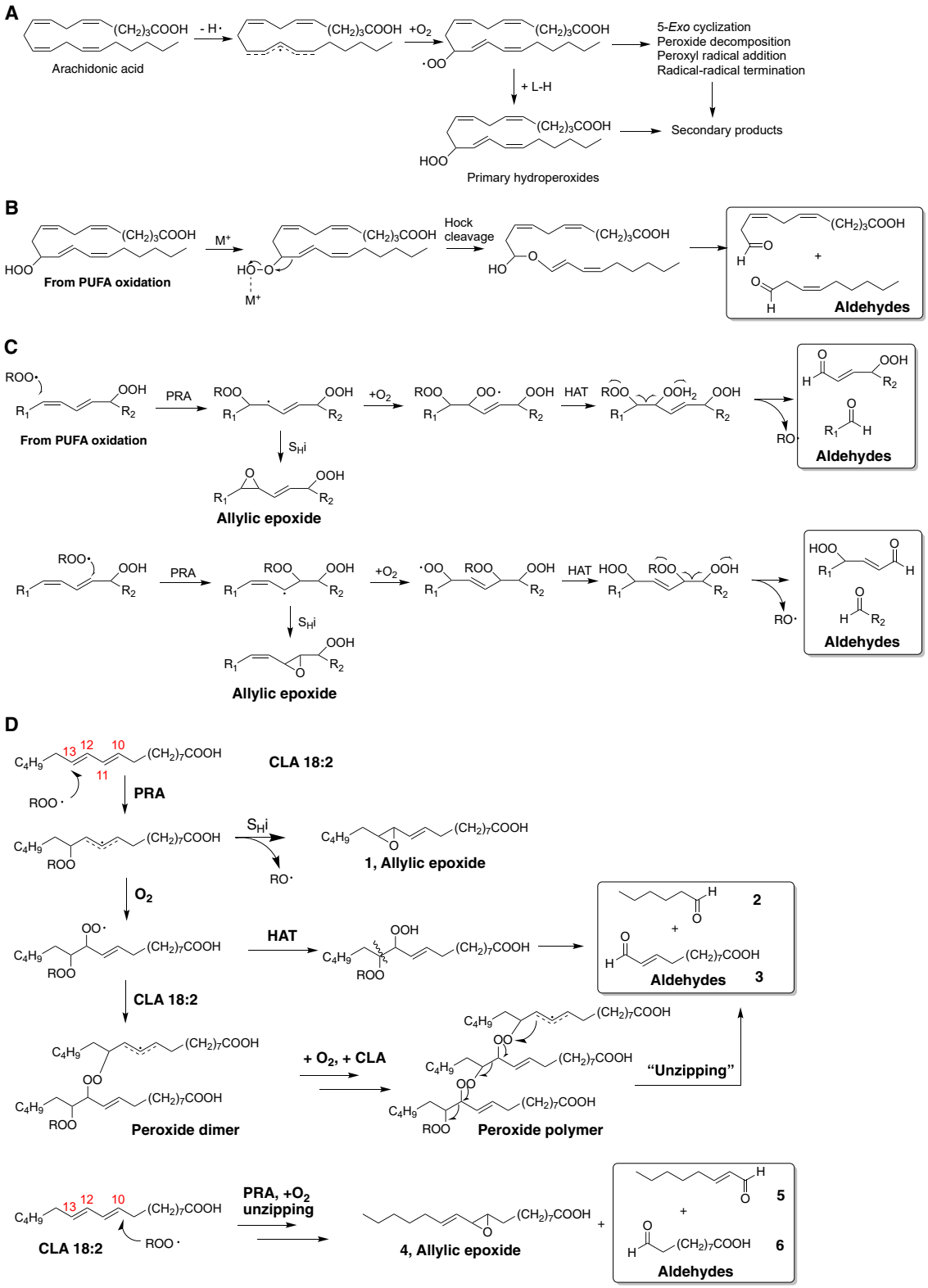

Figure 3. Comparison of products formed from HAT and PRA reaction mechanisms

(A) Primary products formed from HAT reactions.

(B–D) Mechanisms for the formation of truncated lipids via C–C cleavage of hydroperoxides or PRA-derived peroxide dimers or polymers.

70% more HNE, while CL 18:2/18:2/18:2/18:2 generated 150% more HNE than PLPC, suggesting that increased PRA reactions significantly enhanced the formation of HNE.[31] Furthermore, Schneider et al. identified a series of allylic epoxide from the autoxidation of 15S-HETE and 15S-HPETE, providing further support to the PRA mechanism.[30]

The third mechanism is the "unzipping" reactions of peroxide oligomers or polymers formed from a series of PRA reactions, as shown in Figure 3D. One classic peroxyl polymerization reaction is the formation of peroxide polymers from the free radical oxidation of styrene.[32,33] "Unzipping" of the styrene peroxide polymers gave an epoxide and a series of formaldehyde and benzaldehyde. Conjugated PUFAs readily undergo such peroxyl polymerization reactions. The unzipping of the peroxide polymers would lead to the aldehydes shown in Figure 3D, depending on the site of the PRA reactions.[16] Peroxide dimers and polymers have been previously identified as the major oxidation products of conjugated PUFAs,[34–37] and volatile aldehyde compounds have also been detected in CLA 18:2-rich oil and purified triacylglycerides (TAGs),[38] providing support to the proposed mechanisms. In our recent work, we successfully identified the volatile aldehydes 2 and 5 by trapping the oxidation products from conjugated PUFAs with 2,4-dinitrophenylhydrazine,[16] providing further support. Overall, the PRA mechanism favors C–C cleavage of PUFAs, and thus truncated lipids, more than the HAT mechanism. Indeed, it was found that the relative levels of total free aldehydes and α,β-unsaturated aldehydes formed from CLA 18:2 oxidation are 1.6 and 3.0 times those formed from nonconjugated LA 18:2, respectively,[16] supporting that the PRA mechanism led to more C–C cleavage and aldehyde formation than the HAT mechanism.

DIFFERENT FACTORS THAT AFFECT FERROPTOSIS THROUGH REGULATING LIPID PEROXIDATION

Regulation of ferroptosis can be viewed as shifting the balance between prooxidative and antioxidative processes.[10,39,40] Ferroptosis is characterized by three hallmarks, all of which are related to the promotion of lipid peroxidation (Figure 4). First, ferroptosis is iron dependent. In the 2012 study, Dixon et al. observed that only the addition of exogenous iron sources, but not other metal ions, could potentiate erastin-induced lethality and that the iron chelator deferoxamine could suppress cell death.[5] Thus, these observations led them to name this form of cell death ferroptosis. It has been suggested that iron plays multiple roles in the promotion of ferroptosis. For example, free intracellular iron can initiate nonenzymatic lipid peroxidation by catalyzing the decomposition of peroxides through "Fenton chemistry."[41] In these redox reactions, labile ferrous iron can react with lipid hydroperoxide, forming highly reactive alkoxyl radicals that can initiate and propagate the lipid-free radical chain oxidation. Indeed, the degradation of the iron storage protein ferritin via ferritinophagy, an autophagic process, leads to increased cytosolic labile iron levels and ferroptosis sensitivity.[42,43] In some contexts, ferroptosis can also be driven by iron-containing enzymes, such as lipoxygenases (LOXs) and P450s, and Fe–S cluster-containing energy-producing protein complexes that can lead to the generation of cellular reactive oxygen species (ROS).[44]

The second hallmark is its dependency on the cellular antioxidant systems against lipid peroxidation. As discussed above, the indirect and direct inhibition of the antioxidant enzyme GPX4 by erastin and RSL3 in cancer cells can lead to the accumulation of lipid peroxidation and, subsequently, ferroptosis.[5,6] On the other hand, radical-trapping antioxidants (RTAs), such as aryl amines (i.e., ferrostatin-1 and

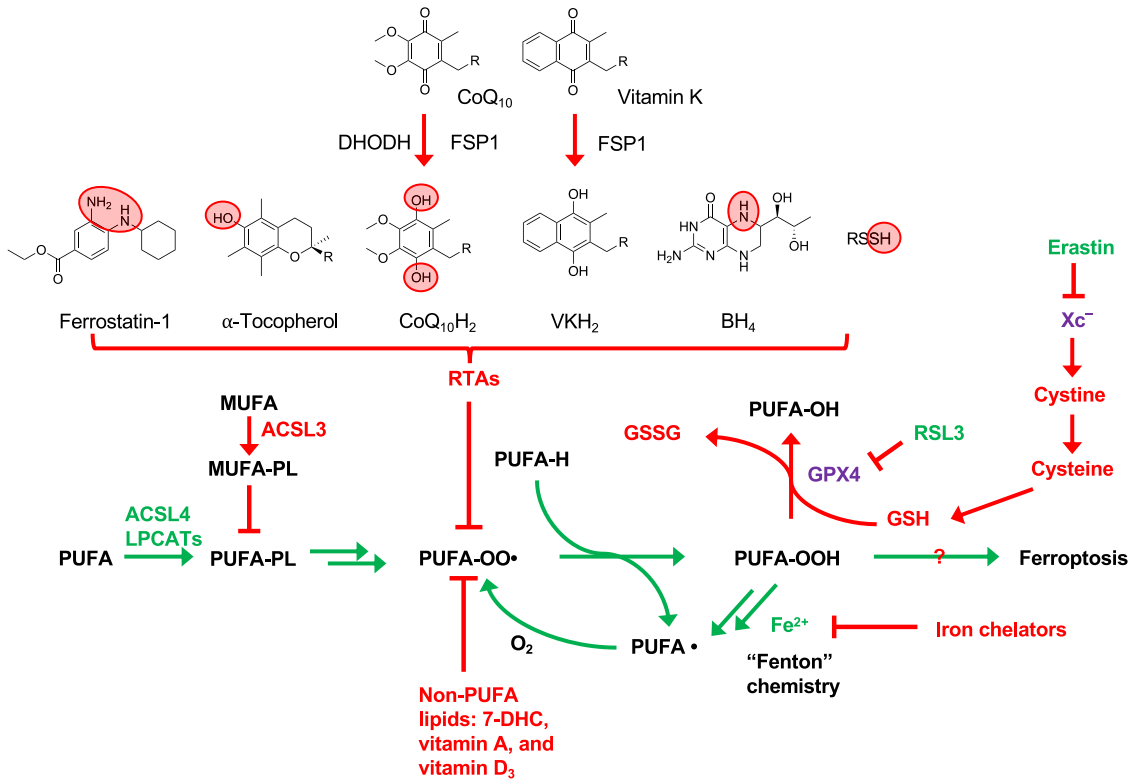

Figure 4. Schematic illustration of regulatory pathways of ferroptosis

Green depicts ferroptosis-driving processes, while red depicts inhibiting processes. RTA, radical-trapping antioxidants; FSP1, ferroptosis suppressor protein 1; GSH, glutathione; GSSG, glutathione dimer; BH_4, tetrahydrobiopterin; VKH_2, reduced vitamin K; RSSH, hydropersulfide; PL, phospholipids.

liproxstatin-1) and α-tocopherol, suppress ferroptosis as they terminate the free radical chain oxidation. Additional ferroptosis-suppressing systems other than GPX4 have been identified recently. Notably, these systems are important for the synthesis of endogenous RTAs, such as the reduced forms of coenzyme Q_{10} ($CoQ_{10}H_2$) and vitamin K (VKH_2),[45–47] both of which are generated by ferroptosis suppressor protein 1 (FSP1), tetrahydrobiopterin (BH_4),[48,49] and hydropersulfides.[50,51] In short, when abundant exogenous or endogenous RTAs are present, lipid peroxidation is suppressed, and ferroptosis is inhibited. In contrast, the loss of function of the antioxidant networks leads to the accumulation of lipid peroxidation and promotes ferroptosis.

The third hallmark is its dependency on the autoxidation of esterified PUFAs in lipid membranes. For PUFAs to exert their lethality, they must be incorporated into the membrane phospholipids. The enzymes involved in the activation and incorporation of PUFAs into membrane phospholipids, such as acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3), play essential roles in the induction of ferroptosis. Indeed, the deletion or inactivation of ACSL4 and LPCAT3 promotes a ferroptosis-resistant state,[8,52–54] and the overexpression of ACSL4 sensitizes cells to ferroptosis.[8] However, it should be noted that ACSL4 was found to be more important to ferroptosis induced by direct GPX4 inhibition than cysteine deprivation.[55] There are two isoforms of ACSL4: one isoform localizes to the inner plasma membrane and, to a lesser extent, cytosol, while the other isoform localizes to the endoplasmic reticulum (ER) and the lipid droplet.[56] Although the final event of ferroptosis is the breakage of the plasma

membrane due to lipid peroxidation, the initiation site resides inside the cell. ER is rich in PUFAs and has been thus suggested to be the most important site of lipid peroxidation during ferroptosis.[10,57] Indeed, lipid hydroperoxides were shown to accumulate predominantly in the ER compartment in RSL3-treated cells.[53] More recently, using Raman imaging, Krusenstiern et al. demonstrated that although lipid peroxidation at various subcellular organelles can induce ferroptosis, the ER is the most important site of lipid peroxidation.[58]

For some time, the enzymatic lipid peroxidation of PUFAs catalyzed by LOXs was considered the driver of ferroptosis. However, it has been found that LOX functions in ferroptosis are context dependent and are more important to erastin-induced than RSL3-induced ferroptosis.[7] Instead, it has been shown that while LOX can contribute to the ferroptosis sensitivity by converting esterified PUFAs into lipid hydroperoxides that participate in the ferroptosis initiation phase via the Fenton reactions, nonenzymatic reactions are the key driver of the propagation of lipid peroxidation in the lipid membrane leading to ferroptosis.[9]

As mentioned above, not all lipid peroxidation, such as 7-DHC, would lead to ferroptosis. We further found that vitamins A and D_3, although highly oxidizable, as shown in Figure 2, protect cancer cells from undergoing ferroptosis.[16] We note that peroxidation k_p values of 7-DHC, vitamin A, and vitamin D_3 (2,260, 5,656, and 1,031 $M^{-1}s^{-1}$, respectively) are all much larger than those of nonconjugated PUFAs (197 $M^{-1}s^{-1}$ for AA 20:4), but the rate constants measured are between linoleate peroxyl radical and each respective lipid instead of between the isoprenoid (7-DHC, vitamin A, and vitamin D_3)-derived peroxyl radicals and another lipid. One possible explanation for the discrepancy between their high reactivities and ferroptosis induction is that oxidation products of phospholipids lead to more membrane instability than products of 7-DHC, vitamin A, and vitamin D_3 and that the highly reactive isoprenoids spare phospholipids from being oxidized. Another possible explanation is that the termination rate constants of these radicals could be much larger than those derived from PUFAs, thus decreasing the overall oxidation rates, which are inversely proportional to the square root of the termination rate constant.[1] These results suggest that only peroxidation of membrane PUFAs is necessary for ferroptosis.

Therefore, PUFAs are incorporated into membrane phospholipids via the actions of ACSL4 and LPCAT3, which predisposes the cells to ferroptosis. Then, during ferroptosis, when the cellular antioxidant networks are impaired, the PUFA tails of membrane phospholipids undergo lipid peroxidation, leading to the degradation of PUFA-containing lipids and the accumulation of lipid oxidation products.[48,53] Once the pool of oxidation products reaches a certain threshold, ferroptosis is induced.

THE MISSING LINKS BETWEEN LIPID PEROXIDATION AND CELL DEATH

The final event of ferroptosis is the breakage of the lipid membrane, but the detailed mechanism through which lipid peroxidation mediates this process remains incomplete (Figure 5). Previously, oxidized lipid tails were shown to undergo reorientation to protrude into the aqueous phase, leading to a reduction in membrane thickness and changes in the lipid bilayer's physical properties.[59] In the context of ferroptosis, molecular dynamic simulation of lipid membrane undergoing peroxidation showed that increased lipid peroxidation in the membrane led to increased membrane thinning and curvature as well as membrane damage through micelle formation.[24] More

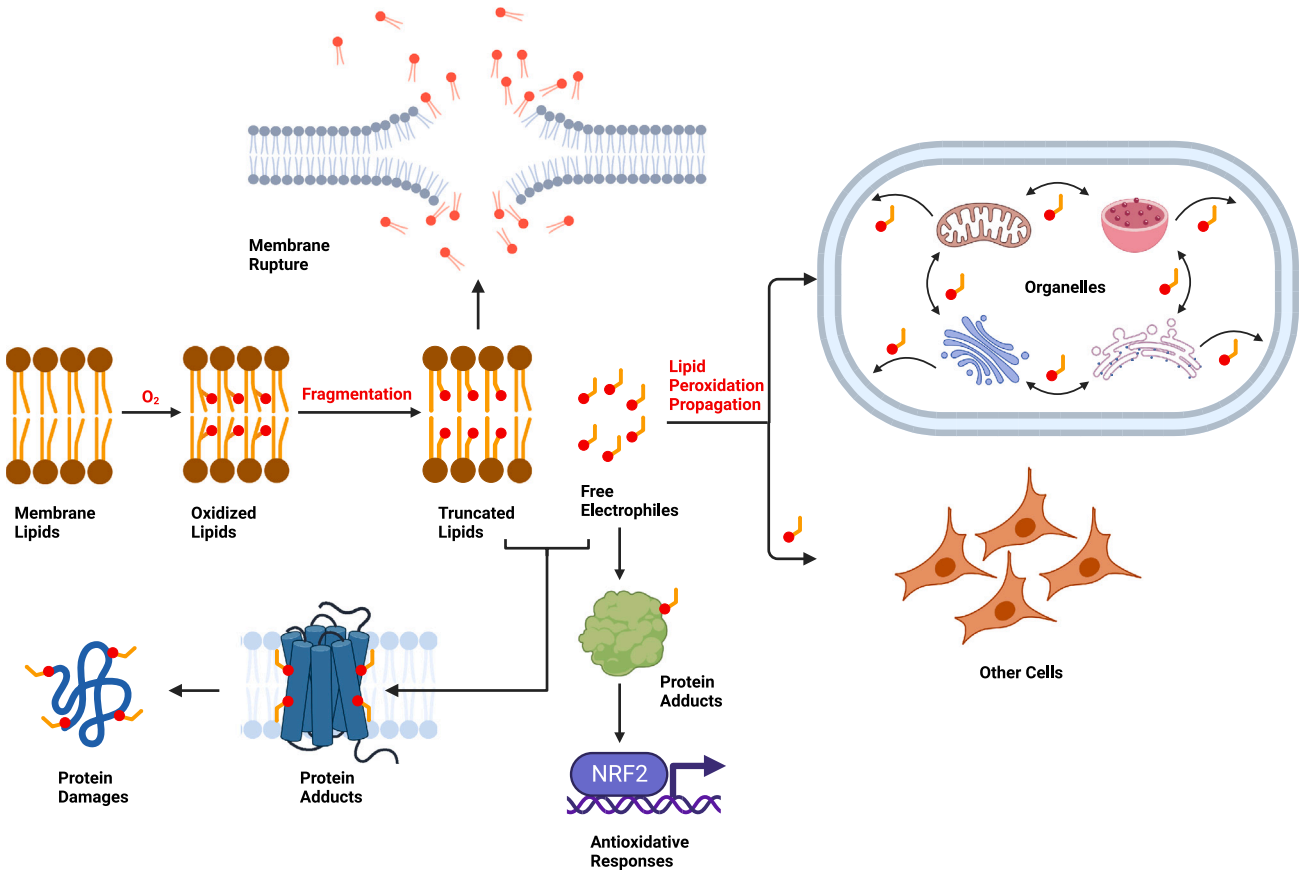

Figure 5. Proposed roles of truncated lipids and lipid electrophiles derived from lipid peroxidation in ferroptosis
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recently, it was determined that pore formation, increased cell swelling and calcium influx, and, eventually, cell rupture are the late events in the cell death progress of ferroptosis.[60,61] Importantly, pore formation on lipid membrane was only observed for lipid-derived aldehydes, not lipid peroxides, owing to their shorter and highly mobile tails.[62] Recently, Angeli et al. found that the accumulation of autoxidation-derived truncated lipids, rather than lipid hydroperoxides, is critical for ferroptosis.[20] Furthermore, Van Kessel et al. found that impaired detoxification of lipid-derived electrophiles is a hallmark of ferroptosis using live-cell imaging.[63] Thus, the formation of lipid electrophiles from PUFA peroxidation is critical for the execution of ferroptosis. Because PRA reactions favor C–C cleavage of PUFAs and the formation of lipid electrophiles, these studies highlight the importance of the PRA mechanism during ferroptosis. Interestingly, Kraft et al. reported that phospholipids with two PUFA chains are particularly relevant to ferroptosis in cancer cells,[48] supporting the importance of the PRA mechanism in ferroptosis since autoxidation of lipids with two PUFAs favors C–C cleavage through the interchain PRA reaction.[31]

The importance of the PRA mechanism is further demonstrated in ferroptosis induced by conjugated PUFAs.[17,64] Nonconjugated PUFAs, such as AA, can potentiate ferroptosis induction by canonical inducers, such as erastin and RSL3, but cannot induce ferroptosis by themselves. However, it was recently shown that conjugated PUFA 18:3 (CLA 18:3) can induce ferroptosis in cancer cells in the absence of other inducers through a GPX4-independent mechanism.[17,64] Even before these

recent studies, conjugated PUFAs have long been shown to exert anticarcinogenic and anticancer effects.[65–67] Parinaric acid, an 18:4 conjugated fatty acid, is >25 times more cytotoxic to cancer cells than corresponding nonconjugated fatty acids, and the antioxidant butylated hydroxytoluene abolishes its cytotoxicity, indicating a lipid-peroxidation-mediated cell death mechanism.[65] In recent work, we validated that CLA 18:3 indeed induces ferroptosis by itself.[16] Furthermore, we found that CLA 18:2 is much more potent in increasing the toxicity of RSL3 in cancer cell lines than any nonconjugated PUFAs. The difference in potency of CLA 18:2 and nonconjugated PUFAs can, in part, be attributed to their difference in autoxidation mechanism and kinetics. CLA 18:3 undergoes autoxidation predominantly through the PRA mechanism (k_{add} = 876 $M^{-1}s^{-1}$ vs. k_H = 359 $M^{-1}s^{-1}$), and CLA 18:2 undergoes autoxidation via both HAT and PRA mechanisms (k_H = 57 M^{-1} s^{-1} and k_{add} = 61 M^{-1} s^{-1}), while nonconjugated PUFAs do not have any contribution from the PRA mechanism. These results suggest that the high reactivity of CLAs toward PRA and the PRA-derived oxidation products (Figure 4D) are likely underlying their high potency in inducing or potentiating ferroptosis.

It is important to note that lipid electrophiles formed from C–C cleavage of lipid peroxides can remain attached to the phospholipid backbone (truncated phospholipids) or become freely diffusible, such as 4-HNE, depending on the direction of the fragmentation (Figure 3). These electrophiles can cause protein damage by covalently modifying the nucleophilic residues,[68] but they can also induce antioxidative responses at low levels, such as the activation of the Keap1-Nrf2 pathway.[69] Previously, ferroptosis was shown to propagate between neighboring cells independently from cell rupture.[60] Furthermore, Nishizawa et al. found that the secretome of ferroptotic cells can propagate cell death and suggested that lipid peroxides in the secretome may be the responsible component. While oxidized lipids can be released from membrane phospholipids via phospholipases,[70,71] we propose that the released freely diffusible lipid electrophiles containing a peroxide group could also serve the role of intercellular propagation of lipid peroxidation, as the peroxide group can react with iron to initiate new lipid peroxidation reactions (Figure 5).

CONCLUSIONS AND REMAINING QUESTIONS

Ferroptosis is driven by lipid peroxidation, and thus factors that affect each step of the free radical chain oxidation sequence (Figure 1) could impact ferroptosis. Indeed, the biological regulation of ferroptosis largely involves the regulation of the antioxidative systems that either reduce hydroperoxide (GPX4) or produce RTAs ($CoQ_{10}H_2$, VKH_2, hydropersulfides, etc.). This perspective aims to bring new outlooks on how different lipid peroxidation propagation mechanisms (HAT and PRA) and derived products could contribute differently to ferroptosis. Previous work on ferroptosis focuses on the role of lipid hydroperoxides, which are mostly derived from the HAT mechanism, while products derived from the PRA mechanism have been largely overlooked. The major differences in the product profiles between the HAT and PRA mechanisms are the preferential formation of peroxide dimers and oligomers from the PRA mechanism. The decomposition of such dimers and oligomers favors the formation of C–C cleavage products, leading to truncated phospholipids and freely diffusible lipid electrophiles. Such lipid electrophiles can form adducts with proteins, which have been observed in the context of ferroptosis,[72,73] potentially leading to protein damage and aggregation. The importance of the PRA mechanism is highlighted by the induction of ferroptosis by CLA 18:3 in a GPX4-independent manner. We further discussed that not all highly oxidizable lipids, such as

7-DHC, vitamin A, and vitamin D_3, potentiate ferroptosis. Instead, they inhibit cell death, supporting the importance of membrane phospholipids in ferroptosis.

Several questions bridging lipid peroxidation and the eventual cell death remain (Figure 5). First, does the protein damage or aggregation induced by membrane-bound or free lipid electrophiles contribute to the membrane rupture? Second, do the lipid peroxide dimers and oligomers in cells directly contribute to changes in membrane properties and eventual rupture? Third, do freely diffusible lipid electrophiles contribute to the propagation of lipid peroxidation between organelles or cells? We are hopeful that these questions can be answered with new imaging and analytical techniques and chemical tools in the near future.

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AUTHOR CONTRIBUTIONS

L.X. conceptualized the manuscript. Both Q.D. and L.X. drafted and revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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