Determining the role of peroxidation of subcellular lipid membranes in ferroptosis

Ferroptosis can be induced by lipid peroxidation in various subcellular membranes, including the endoplasmic reticulum (ER), mitochondria and lysosomes. By studying the subcellular distribution of ferroptosis-modulating fatty acids, we observed that the ER is a key initial site of peroxidation, followed by the plasma membrane, whereas other organelles are not as critical for ferroptosis.

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The question

Ferroptosis is a non-apoptotic form of cell death that is driven by iron-dependent lipid peroxidation. Ferroptosis has been implicated in diseases including neurodegeneration and ischemia-reperfusion injury, and initiation of ferroptosis is a promising avenue for cancer treatment[1]. There remain questions about the different players and stages of ferroptotic death. Although it is known that lipid peroxidation in membranes drives ferroptosis[1], whether certain membranes are essential and at what point they are oxidized has been unclear. A deeper understanding of the mechanisms that drive ferroptosis will facilitate the development of new medicines and biomarkers and strengthen our insight into how cells die in diverse diseases.

The observation

In this project, we sought to elucidate the subcellular dynamics of lipid peroxidation in ferroptosis. We approached this question by exploring the subcellular distribution of different ferroptosis-modulating compounds: fatty acids that make cells susceptible to or protect against ferroptosis, and $FINO_2$, a 1,2-dioxolane that contains an endoperoxide moiety that induces ferroptosis[2]. Polyunsaturated fatty acids (PUFAs), such as arachidonic acid and docosahexaenoic acid (DHA), are excellent substrates for peroxidation and, therefore, drive ferroptosis, whereas monounsaturated fatty acids (MUFAs), such as oleic acid, block ferroptosis through an unknown mechanism[1]. We used both Raman microscopy and confocal fluorescence microscopy to explore the subcellular distributions of these compounds (Fig. 1). The distribution of ferroptosis-modulating fatty acids helped to identify which membranes are likely to be key targets of lipid peroxidation. By targeting $FINO_2$ to different subcellular membranes, we evaluated whether peroxidation of certain membranes is a prerequisite for initiating ferroptosis. Finally, we used a fluorescent fatty acid analog that detects lipid peroxides (BODIPY 581/591 C11) to capture the evolution of lipid peroxidation in different cellular membranes over time.

Our findings converged on common key subcellular sites. We observed both PUFAs and MUFAs primarily accumulating in the ER, with some incorporation in the plasma membrane and a small amount in the mitochondria. We found that modulating the fatty acid content of these sites by addition of MUFAs or PUFAs to the medium influenced cell sensitivity to ferroptosis. These results pointed to the ER and plasma membrane as essential sites of peroxidation. We then explored the distribution of direct peroxide-forming ferroptosis inducer $FINO_2$ and found that it was targeted to the ER. However, re-localizing $FINO_2$-like compounds to mitochondria or lysosomes also induced ferroptosis, and we were surprised to discover that ferroptosis could be induced by $FINO_2$ in any of these membranes. Finally, we observed that for all four canonical classes of ferroptosis inducers (that is, inhibitors of the cystine/glutamate transporter, which deplete glutathione, GPX4 (phospholipid hydroperoxide glutathione peroxidase) inhibitors and degraders, which block repair of lipid peroxides, and endoperoxides, which directly trigger lipid peroxidation), ER peroxidation occurred before plasma membrane or mitochondrial peroxidation. As mitochondria are not required for all types of ferroptosis[3,4], these findings again point to the ER followed by the plasma membrane as key sites of lipid peroxidation that drive ferroptotic cell death.

The interpretation

The two important findings in this work are that lipid peroxidation can be initiated in several different organelles and results in ferroptosis, and that the ER is an essential early site of lipid peroxidation for ferroptosis-inducing compounds. These findings indicate that the ER should be considered a crucial target for compounds that block ferroptosis, and that dysregulation of lipid content and peroxidation in the ER is likely to be an important determinant of pathological cell death in disease[5].

Although we were able to visualize compounds at a subcellular level, we were limited by the tools and technology that were available. Selectively modulating the size and lipid content of these organelles individually as well as more sophisticated tools for measuring live lipid peroxidation would enable a deeper understanding of these mechanisms. Ultimately, some membranes, such as those of the ER and plasma membrane, are difficult or impossible to eliminate, and require alternative techniques to evaluate their involvement.

One question that emerges from this work is how lipid peroxidation spreads from membrane to membrane within cells, or whether it even spreads at all. It will be interesting to determine whether each membrane is oxidized independently or whether there is a role for lipid trafficking in the spread of oxidation.

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EXPERT OPINION

"von Krusenstiern and colleagues use conventional fluorescence and Raman scattering imaging to understand which cellular membranes are peroxidized during ferroptosis. They conclude that the ER is the primary site of lipid peroxidation and the plasma membrane is a secondary site; mitochondrial membranes can also be sites of ferroptosis, but protecting the ER from lipid peroxidation is sufficient to block mitochondria-initiated ferroptosis. This study is of high quality and will be important to the field." **An anonymous reviewer.**

FIGURE

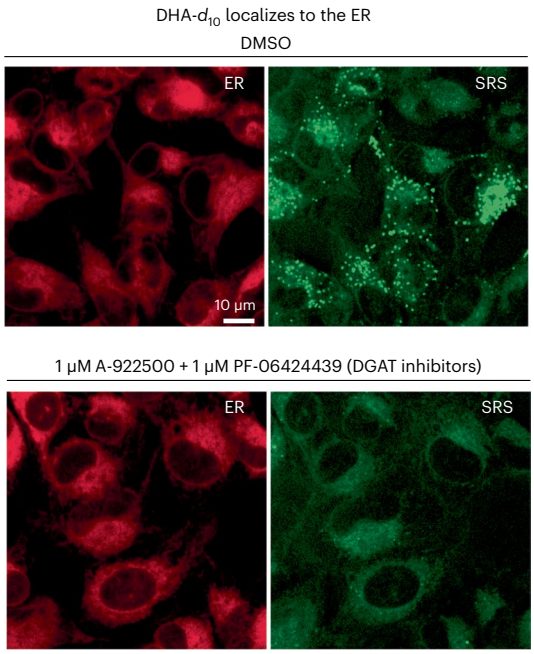

Fig. 1 | Ferroptosis can be induced in different membranes, but the ER is a key initial site of peroxidation. Fluorescence microscopy imaging (left) and stimulated Raman scattering (SRS) imaging (right) of cells treated with deuterated (in which hydrogen has been replaced by deuterium) DHA (DHA-d_{10}) show a DHA-d_{10} distribution that is consistent with ER localization. Treatment with diglyceride acyltransferase (DGAT) inhibitors results in elimination of lipid droplets. DMSO, dimethyl sulfoxide. © 2023, von Krusenstiern, A. N. et al.

BEHIND THE PAPER

The questions of where lipid peroxidation occurs in ferroptosis and which membranes are required have been longstanding in the field. This work arose out of two different collaborations that led all our groups in the same direction. With Dr. Wei Min's group, we had been exploring the distribution of fatty acids throughout different cell membranes. With Dr. Keith Woerpel's group, we redistributed $FINO_2$ to different cellular locales to determine whether ferroptosis could still be induced. These projects came together in a meeting in which we realized that all our work pointed to the ER as a central site of lipid peroxidation. Once combined, these projects morphed into a two-pronged approach that enabled us to build a more convincing case. We were also excited to show the utility of evaluating the structure–activity–distribution relationship of multiple chemical probes using two different imaging techniques. **B.R.S.**

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FROM THE EDITOR

"This work by von Krusenstiern et al. stands out because the smart use of imaging methods to visualize the lipid and ferroptosis modulators enables the monitoring of the lipid flow and the identification of subcellular sites essential for ferroptosis induction. These findings promote the mechanistic understanding of ferroptosis and provide clues for future drug development targeting this biological process." **Editorial Team**, ***Nature Chemical Biology.***