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FIRST REACTIONS

Lipoxygenases—Killers against Their Will?

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Phospholipid autoxidation is required for ferroptosis.

While phospholipid oxidation is the key event during ferroptosis, mechanisms responsible for this oxidation during ferroptosis have been a matter of intense debate regarding its source and role in ferroptosis. Here, Shah et al. provide compelling evidence supporting a role for phospholipid autoxidation as an essential requirement for ferroptosis execution, and suggest that enzyme-driven phospholipid oxidation via lipoxygenases may only lower the required threshold to trigger the ferroptotic process rather than being an active player.[1]

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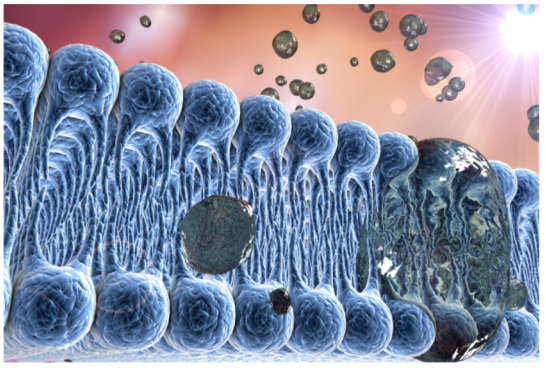
Ferroptosis has been linked to diseases ranging from tissue ischemia/reperfusion injury and neurodegeneration to immunity and cancer.[2] We know today that ferroptosis is fundamentally linked to cysteine and glutathione (GSH) metabolism, as reflected by the absolute requirement of glutathione peroxidase 4 (GPX4) to use GSH or other cysteine-derived thiols to efficiently detoxify peroxidized lipids in membranes.[3] It is important to note that GPX4 acts at the level of peroxides, reducing them to their corresponding alcohols or water in a two-electron-reduction mechanism (Figure 1). This enzymatic activity is also believed to be essential to suppress the activity of the (phospho)lipid peroxide generating lipoxygenases,[4] which can only work when their non-heme bound iron is oxidized to iron(III) by a peroxide. Counterintuitively, it was also demonstrated that vitamin E, a radical trapping antioxidant and



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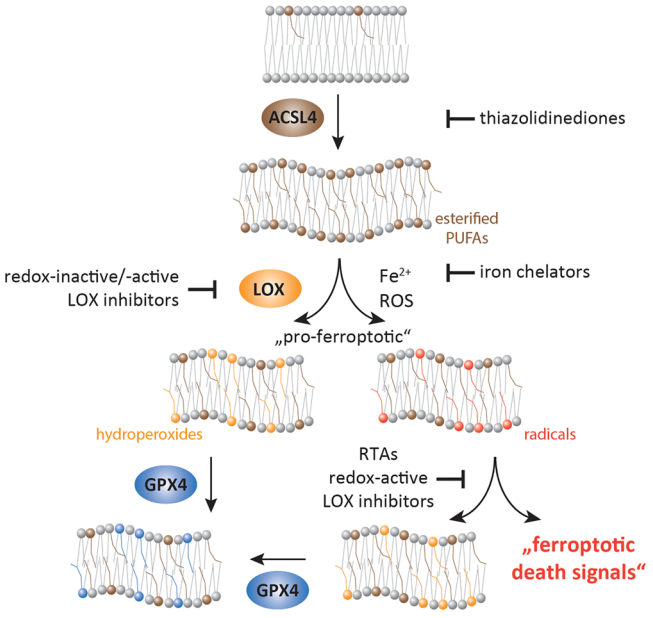

Figure 1. Molecular interplay between pro- and antiferroptotic players in shaping the cellular phospholipidome. ACSL4: acyl-CoA synthetase long chain family member 4. GPX4: glutathione peroxidase 4. LOX: lipoxygenase. ROS: reactive oxygen species. RTA: radical trapping agents.

classical one-electron reductant, could fully prevent GPX4 knockout-induced cell death.[4] These early observations posed an intriguing but difficult to rationalize phenomenon, that is, how a one-electron reductant could functionally compensate the absence of a two-electron reductant. These considerations

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thus raised speculations of a latent novel function of vitamin E, by which it might directly act on lipoxygenases.[5] Yet, an opposing mechanism driving the process of lipid peroxidation has been proposed, whereby the labile iron pool in the cell would be the causative agent.[6] Now the work of Shah et al. provides a potential unifying mechanism bringing together these two apparent discrepancies. By challenging what is currently being proposed, the Pratt group infers that lipoxygenases do not play a causative role during the death process. That is, upon triggering ferroptosis acutely blocking lipoxygenase will not prevent cells from dying. Reflecting the notion that in order to trigger ferroptosis, a "seed" peroxide is required which upon reacting with iron will ignite a chain reaction leading to the accumulation of more oxidized lipids. This further supports a scenario whereby increased lipoxygenase activity would only increase the number of "seeds" but will not take part in igniting them. Additionally, the authors also provide evidence that most of the studies carried out so far using lipoxygenase inhibitors were misinterpreted based on their findings that most so-called lipoxygenase inhibitors typically used to inhibit ferroptosis block autoxidation of (phospho)lipids similar to vitamin E, rather than by inhibiting lipoxygenases. Thus, during normal function, lipoxygenases only sensitize cells to ferroptosis by facilitating the iron-mediated one-electron reduction of preformed phospholipid hydroperoxides into peroxyl radical in order to to generate lipid mediators used by a wide array of cellular functions. In essence, Pratt and colleagues suggest that cells can cope with increased levels of phospholipid hydroperoxides as long as they are kept in check by radical trapping antioxidants, such as vitamin E and the ferroptosis inhibitors ferrostatin-1 and liproxstatin-1. These effects are not merely artifacts of cell culture, as reflected by the *in vivo* situation, where vitamin E is able to compensate GPX4 deficiency in specific cellular types, such as T-cells, hepatocytes, and endothelial cells.[7,8]

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Despite the still missing unequivocal proof that there is an indispensable role for lipoxygenases in ferroptosis (perhaps to be provided by gene knockout studies), the worked carried out by Pratt and co-workers not only helps us to reconsider the role of lipoxygenases and iron as culprits in ferroptosis but also paves the way for new concepts in cancer biology. This is particularly relevant in light of the recent finding that cancer cells become highly sensitive to GPX4 inhibition by transitioning to a mesenchymal state, though we do not know why.[9] It could be due to the fact that lipoxygenase activity is increased in these cellular states. Perhaps instead they switch their phospholipid profile—potentially through acyl-CoA synthetase long chain family member 4 (ACSL4)[10]—thus providing lipid substrates for lipoxygenases. If so, why would they need this? As such, at this stage, it is tempting to speculate that a mesenchymal state is associated with increased production of lipoxygenase-derived eicosanoids (fatty acid signaling molecules). Eicosanoids can, among other things, subvert the innate immune response and/or lead to neovascularization, all critical events during cancer progression.[11] Therefore, it is becoming evident that lipoxygenases are by no means designed by Nature as weapons to kill cells, but rather, they generate a liability during their "normal" function that can be therapeutically exploited in particular cases.

You've got to believe I'm innocent. If you don't, take my job.

Richard M. Nixon

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