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Abstract

Ferroptosis is an iron-dependent mode of cell death driven by lipid peroxidation, capable of explosively propagating through a field of cells. Two studies now explore the mechanisms underlying ferroptotic cell death and its spread as well as its possible *in vivo* significance, shedding light on some of the burning questions surrounding ferroptosis.

Main text

If a cell were an instruction manual, the very last paragraph would delineate a bewildering array of options for its disposal[1]. For example, the programmed, inflammatory 'silent' form of cell death, apoptosis, could be thought of as the equivalent of an instruction to carefully fold and shred the manual before sweeping it tidily into the recycling bin[2]. This is in stark contrast to the highly inflammatory necrosis, which is thought to be unregulated and could be likened to scrunching up the manual into a paper ball and throwing it haphazardly across the room[2]. The difference between unregulated necrosis and regulated necrosis, such as necroptosis) is a subtle, but important, distinction[3]. Nevertheless, many forms of regulated necrosis whereby cells deliberately instigate a necrotic demise, have been identified[1]. It is becoming increasingly clear that cells contain multiple pathways by which to die to impart one last signal or message to those cells it leaves behind before its ultimate departure[4].

Ferroptosis is an iron-dependent form of cell death arising from the accumulation of lipid peroxides[5, 6]. [AU, please remove this as suggested in the last comments. This is for journal style preferences.] Increasing lipid peroxidation can initiate a chain reaction that can induce ferroptosis. Furthermore, once ignited, ferroptosis can spread between cells as a rapidly propagating wave[7]. Key outstanding questions regarding ferroptosis include whether it is truly an 'instructed' or regulated mode of cell death or simply an inherent outcome of a cell's lipid biochemistry that can only be induced unintentionally or experimentally. Related to this important question is whether ferroptosis or its ability to propagate has any biological role *in vivo.* In this issue of *Nature Cell Biology*, Riegman. *et al.* and Katikaneni. *et al.,* explore such possibilities *in vitro* and *in vivo* in two complementary studies. They demonstrate comparable mechanisms by which rapid spatial propagation of lipid peroxide signals promote neighbouring cells to either relay a long-range alarm to immune cells or induce and further spread ferroptosis[8, 9].

Taking an elegant *in vitro* approach, Riegman *et al.*[8] successfully break down the process of ferroptotic cell death and its wildfire spread through neighbouring cells into discrete steps[8].

They first mathematically defined the wave-like propagation frequently observed when ferroptosis is induced *in vitro*. This allowed them to quantitatively distinguish propagating death from cells dying randomly and autonomously from one another by other means (e.g. apoptosis) or by independent ferroptosis, which was not influenced by any other ferroptotic event. Strikingly, this revealed that the now well-established collection of ferroptotic inducing agents were actually inducing different patterns of ferroptosis[10]. Agents such as erastin and others instigated ferroptosis that spread through neighbouring cells as a wave, in an iron and lipid peroxidation dependent manner. However, intriguingly, direct inhibition of GPX4 (*the* ferroptosis-defining antagonistic enzyme that counteracts lipid peroxidation[1, 11]) induced ferroptosis, but in the absence of any propagation between neighbouring cells. This finding goes some way to explain why propagation of ferroptosis has been haphazardly observed experimentally. Furthermore, it demonstrates that ferroptotic cell death is a distinct step from the propagation of this cell death through a field of cells and hints that this mode of cell death and/or its spread might be regulated in some way.

The other major finding from Reigman *et al.* is that, like other forms of regulated necrosis (e.g. necroptosis and pyropotosis), ferroptosis requires plasma membrane pore formation[1]. The authors found that pore formation was not required for propagation of ferroptosis through neighbouring cells, suggesting the former drives the lytic event underlying ferroptotic cell death. Furthermore, this work implies a temporal order to the above whereby the ferroptotic cell induces (or in our analogy, ignites) cell death in its neighbours before dying (or in our analogy, burning itself out) via pore-mediated lysis (Figure 1). The actual composition of these pores remains unresolved as does the mechanism of their formation. However, the role of pore formation in ferroptosis hints at an underlying biological process and therefore perhaps a potential point of regulation.

Taken together, this work by Reigman *et al*. suggests that ferroptosis is not a cellular self-combustion, which can uncontrollably set light to and spread through neighbouring cells[8]. It instead implies that ferroptotic cell death is a discrete step from its propagation. Although not definitive, this suggests a route through which regulation might be applied. The finding that direct inhibition of GPX4 induces ferroptosis, but is insufficient to spark propagation to neighbours is puzzling. All ferroptosis-inducing agents known to date act either by directly or indirectly inhibiting the activity of GPX4[10, 11]. It is possible that the depletion of GPX4's cofactor, glutathione, by drugs such as erastin, makes a larger hole in the cells antioxidant defences, which in turn dramatically increases lipid peroxidation to uncontrollable levels. Perhaps, if given a chance, cells induce a deliberate 'controlled-burn' before lipid peroxidation levels reach those that can induce an explosive chain reaction of ferroptotic spread? The insights from Reigman *et al*. raise many more questions and lay the foundations for further mechanistic investigation into this intriguing mode of cell death.

In the second study, Katikaneni *et al.* take an alternative approach and explore the role of lipid peroxidation in driving damage-induced inflammation in the tail fin of zebrafish larvae[9]. They devised a means of microperfusing biological substances directly to larval tail fins, in the absence of wounding. This allowed the authors to directly test the ability of known wound-released factors (e.g. arachidonic acid and H_2O_2) to act as *bona fide* chemoattractants[12, 13]. They found that arachidonic acid alone (but not the stimulation of H_2O_2 production) could draw leukocytes out of the circulation towards the source of

application. Microperfusion of arachidonic acid was sufficient to generate gradients of lipid peroxidation, which were visualised *in vivo* through the use of a biosensor. Such gradients also emanated from the edge of tail fin wounds. Strikingly, these peroxide gradients covered a distance that was an order of magnitude larger than those that can be stably generated by H_2O_2[9]. Furthermore, such gradients were a much better match for the typical distance travelled by leukocytes during their inflammatory recruitment to wounds. Katikaneni et al went on to show that gradients of lipid peroxidation were absent from wounds when H_2O_2 production was inhibited and the ensuing inflammatory response was suppressed. However, the inability of H_2O_2 to attract leukocytes when its production was stimulated via microperfusion, combined with its short range of action, implies that it is not operating as a chemoattractant driving leukocyte recruitment to wounds. Instead Katikaneni *et al*. concluded that H_2O_2 augmented the inflammatory response, akin to throwing fuel on the fire. This finding is consistent with previous studies in *Drosophila* in which H_2O_2 acts as an activation signal, but does not fit the necessary criteria to be considered a chemoattractant for immune cell recruitment to wounds[14, 15]. Katikaneni *et al.* showed that arachidonic acid specifically increased lipid peroxidation *in vivo* and furthermore, leukocytes were able to follow a positive gradient of lipid peroxidation towards its source (e.g. a wound). As such, lipid peroxidation appears to serve as a means of transmitting instructive messages across large distances *in vivo*. Presumably, this is achieved through a self-perpetuating wave of lipid peroxidation between neighbouring cells, exactly as ferroptosis is propagated, albeit at a highly restrained amplitude that does not induce cell death. However, if true, there is no denying that this is metaphorically playing with fire, as, through their use of this volatile lipid biochemistry, cells risk instigating widespread ferroptosis.

Indeed, Katikaneni *et al.* found that in a rare few instances, the application of arachidonic acid by microperfusion did induce a wave of cell death within the larval tail fin, which was highly reminiscent of propagating ferroptosis[9]. The authors acknowledge that this is no more than suggestive and will require much further study to delineate. However, it is tempting to speculate that ferroptosis may be an inevitable, but ultimately necessary risk, associated with utilising lipid peroxidation as a long-range tissue messenger. This can be likened to the use of hilltop signal fires to rapidly send messages across vast distances to alert and recruit defensive forces (i.e. inflammatory leukocytes). However, the harness of fire, here lipid peroxidation, for whichever purpose, always carries with it the danger of starting an out of control wild fire, or in this case a propagating wave of cell death (Figure 2).

Together, these two studies by Riegman *et al.*[8] and Katikaneni *et al.*[9] challenge the oft prevailing view that ferroptosis is merely an accidental cell death arising from the overwhelming accumulation of too much toxic, oxidised lipid[6]. Instead they hint at a subtler, underlying regulation and biological purpose, which is perhaps overshadowed by the explosively propagating ferroptosis usually induced experimentally. Further investigation will be required before such conclusions can be reached, but these papers oblige us to revisit our assumptions regarding ferroptosis. [AU, please remove this. This is for journal style preferences.]

Figure 1. Cell-cell propagation of ferroptosis is a distinct step from ferroptotic cell lysis. (1) Increasing cellular lipid peroxidation induces ferroptosis. (2) This can initiate a chain reaction whereby toxic lipid conversion and ferroptosis spreads to neighbouring cells. (3)

Meanwhile, subsequent pore formation induces cell lysis as an independent event from propagation of ferroptosis through a field of cells.

Figure 2. Lipid peroxidation is utilised as a long-range communication system to relay inflammatory signals to recruit leukocytes (green), despite the risk of inducing catastrophic spread of ferroptosis. Wounding of the larval zebrafish tailfin induces a low level of lipid peroxidation that spreads between neighbouring cells in a wave-like manner to establish a far-reaching tissue gradient. This alerts leukocytes to the tissue damage and guides their inflammatory recruitment. However, uncontrollable levels of lipid peroxidation induce propagating ferroptosis that spread and kills the unwounded surrounding tissue.

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Competing Interests

The authors can confirm that they have no competing interests.