MOLECULAR & CELLULAR ONCOLOGY
2017, VOL. 4, NO. 3, e1302906 (3 pages)
https://doi.org/10.1080/23723556.2017.1302906

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AUTHOR'S VIEW

Ferroptosis-like death in plant cells

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ABSTRACT
Ferroptosis is an iron-dependent, oxidative, non-apoptotic form of cell death initially described in mammalian cells. We recently reported that a ferroptosis-like cell death process can be triggered by heat shock in *Arabidopsis thaliana*. Thus, ferroptosis may be a form of cell death conserved between animals and plants.

ARTICLE HISTORY
Received 28 February 2017
Revised 1 March 2017
Accepted 1 March 2017

KEYWORDS
Arabidopsis; glutathione; iron; reactive oxygen species

In multicellular organisms, regulated cell death plays an essential homeostatic role by eliminating damaged or unwanted cells. Individual cells are eliminated through the activation of specific biochemical pathways. Several pathways have been discovered in animal cells, including the classic caspase-dependent apoptosis pathway and several biochemically distinct non-apoptotic cell death pathways.[1] The core apoptosis pathway is broadly conserved among metazoans.[2] The degree to which any of the known non-apoptotic cell death pathways are conserved throughout evolution, beyond mammals, is generally less clear. Our recent collaborative work, spearheaded by the Pagnussat laboratory, suggests that an iron-dependent, oxidative form of non-apoptotic cell death termed ferroptosis, which to date has only been observed in mammalian cells, could be activated in plant cells.[3] This provides evidence that non-apoptotic cell death pathways may be broadly conserved.

Ferroptosis is characterized by the iron-dependent, oxidative destruction of membrane polyunsaturated fatty acids (PUFAs). Membrane PUFAs are susceptible to spontaneous or enzyme-catalyzed oxidation, resulting in the formation of lipid peroxides (L-OOH). In the presence of iron, lipid peroxides can fragment into toxic lipid radicals (e.g. L-O•) and further reactive lipid breakdown products.[4] Normally, this toxic process is suppressed by the activity of the glutathione-dependent phospholipid hydroperoxidase glutathione peroxidase 4 (GPX4), which reduces L-OOH to non-toxic lipid alcohols (L-OH). Reduced intracellular glutathione inactivates GPX4, leading to increased lipid oxidation and, ultimately, ferroptotic cell death. This death can be blocked with small molecule iron chelators or lipophilic antioxidants, such as ciclopirox (Cpx) and ferrostatin-1 (Fer-1), respectively, which are useful tools that can be used to study this process in diverse contexts (Fig. 1A).

In plants, various cells undergo developmental, homeostatic, or stress-induced cell death, but the biochemical mechanisms responsible for these lethal events are only partially understood.[5] We therefore investigated whether ferroptosis or a ferroptosis-like process might be activated under certain conditions in plants. Using *Arabidopsis thaliana* as a model system, we found that exposure of root hairs to heat shock (55°C, 10 min) triggered cell death that could be suppressed by pretreatment with the canonical ferroptosis inhibitors Cpx and Fer-1[3]. These same inhibitors had no effect on root hair cell death in response to a stronger heat shock (77°C), direct exposure to an oxidizing agent (hydrogen peroxide), or salt stress (high NaCl). Likewise, Cpx and Fer-1 had no effect on programmed cell death in vascular or reproductive tissues, indicating that the type of death observed in response to a 55°C heat shock was unique. Mechanistically, this death exhibited biochemical similarities to ferroptosis, as described in mammalian cells, insofar as cell death was preceded by the depletion of glutathione and increased markers of oxidative stress. As in mammalian cells,[6] cell death could also be rescued by supplementation with deuterated PUFAs, which are more difficult to oxidize than normal PUFAs. These results indicate that a ferroptosis-like form of cell death may be induced in *A. thaliana* cells in response to a specific stress—55°C heat shock.

This work raises several questions regarding the regulation of ferroptosis-like death in plant cells, and the degree of biochemical conservation between ferroptosis and ferroptosis-like cell death in mammalian and *A. thaliana* cells. First, how does 55°C (but not 77°C) heat shock lead to glutathione depletion and ferroptosis-like cell death in *A. thaliana* root hair cells? In mammalian cells, ferroptosis can be triggered by small molecules that inhibit the import of cystine (which is needed to synthesize glutathione) by the heterodimeric cystine/glutamate antiporter system x_c^-, or by direct inhibitors of GPX4[7,8] (Fig. 1A). Plants do not express sequence orthologs of the system x_c^- genes *SLC7A11* and *SLC3A2*, but 55°C heat shock could inhibit other cystine/cysteine uptake pathways or otherwise block *de novo* glutathione synthesis (Fig. 1B). As in mammalian cells, glutathione depletion could inactivate *A. thaliana* glutathione peroxidase enzymes (i.e. AtGPX1–8), leading to lethal oxidative stress. Another possibility is that 55°C heat shock increases protein

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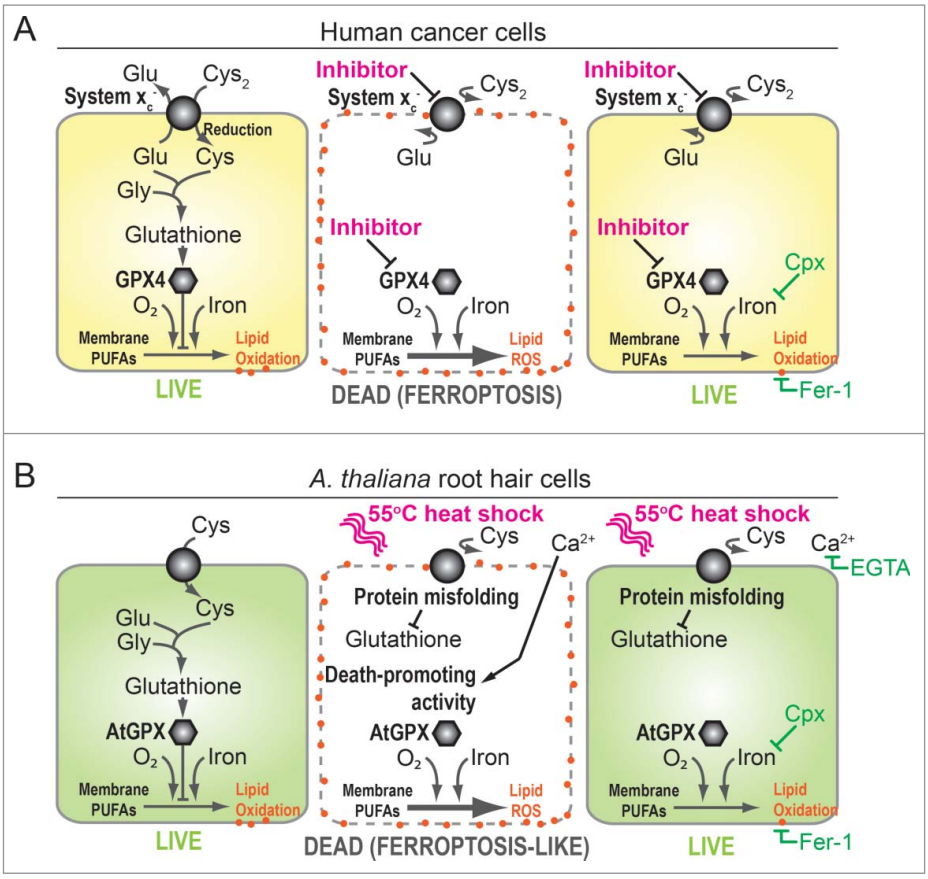

Figure 1. Molecular mechanisms of ferroptosis in mammalian and plant cells. (A, B) Our current understanding of the pathways leading to ferroptosis in mammalian cells and ferroptosis-like cell death in plant cells. In both cell types, glutathione prevents lethal lipid oxidation. Death can be prevented by the iron chelator ciclopirox (Cpx) or the lipophillic antioxidant ferrostatin-1 (Fer-1). (A) In human cancer cells, cystine import via the system x_c^- amino acid antiporter is necessary for glutathione synthesis. Glutathione is used by glutathione peroxidase 4 (GPX4) to suppress lipid oxidation. Inhibitors (pink) of system x_c^- or GPX4 ultimately result in iron-dependent lipid oxidation and ferroptotic cell death. Glu: glutamate, Gly: glycine, Cys_2: cystine, Cys: cysteine. PUFA: polyunsaturated fatty acid. (B) In *Arabidopsis thaliana* root hair cells, 55°C heat shock depletes glutathione by unknown means, possibly leading to inactivation of one or more GPX4 orthologs (AtGPX4). Unlike in human cancer cells, calcium (Ca^{2+}) influx is required for ferroptosis-like cell death. It is unknown whether Ca^{2+} contributes to death by acting upstream or downstream of glutathione depletion in plant cells, or how this influx promotes death. EGTA: ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

misfolding that is reversed in a glutathione-dependent manner. Extensive protein re-folding could deplete glutathione levels below a critical threshold necessary to suppress lipid oxidation, ultimately leading to cell death. Higher heat (i.e., 77°C) may more fully unfold or completely denature proteins in a manner that is irreversible by glutathione, causing death through a distinct mechanism not suppressed by Fer-1 or Cpx. Whether a similar acute heat shock could trigger ferroptosis in mammalian cells is not known. In fact, exposure to a milder but more prolonged heat shock (42.5°C for 8 or 24 h) actually attenuated the subsequent induction of ferroptosis in human cancer cells, possibly via upregulation of specific heat shock proteins.[9] Thus, the relationship between heat shock and the induction of ferroptosis is likely to be dependent on temperature and species.

A second question concerns the role of calcium. In root hair cells, the calcium chelator ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), which chelates extracellular calcium, was found to block ferroptosis-like cell death in response to 55°C heat shock[3] (Fig. 1B). In human cancer cells, chelation of extracellular calcium does not block cell death in response to glutathione depletion,[7] providing one distinction between ferroptosis in these cells and ferroptosis-like death in plant cells. However, in mammalian neuronal-like HT22 cells, extracellular calcium influx is required for cell death downstream of glutathione depletion.[10] Future experiments will be required to determine the role of calcium in response to cell death triggered by 55°C heat shock in root hair cells. Such studies will help establish a more complete picture of the similarities and differences between ferroptosis and ferroptosis-like cell death processes in animal and plant cells.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgment

The authors thank Leslie Magtanong for comments.

Funding

This work was funded by the National Institutes of Health (USA), the Damon Runyon Foundation, and the Hellman Fellows Fund.

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