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Review

Ferroptosis: Death by Lipid Peroxidation

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Ferroptosis is a regulated form of cell death driven by loss of activity of the lipid repair enzyme glutathione peroxidase 4 (GPX4) and subsequent accumulation of lipid-based reactive oxygen species (ROS), particularly lipid hydroperoxides. This form of iron-dependent cell death is genetically, biochemically, and morphologically distinct from other cell death modalities, including apoptosis, unregulated necrosis, and necroptosis. Ferroptosis is regulated by specific pathways and is involved in diverse biological contexts. Here we summarize the discovery of ferroptosis, the mechanism of ferroptosis regulation, and its increasingly appreciated relevance to both normal and pathological physiology.

Trends

Ferroptosis is a regulated, nonapoptotic form of cell death distinct from other cell death modalities.

Loss of GPX4 activity and subsequent accumulation of lipid hydroperoxides executes ferroptosis.

Diffuse large B cell lymphomas and renal cell carcinomas are particularly susceptible to GPX4-regulated ferroptosis.

Inhibition of ferroptosis may represent a promising therapeutic approach for treating pathological conditions such as acute kidney injury.

Discovery of Ferroptosis

Cell death is essential for fundamental physiological processes such as development, immunity, and tissue homeostasis; moreover, cell death is often dysregulated in degenerative and neoplastic diseases. Both apoptotic and nonapoptotic cell death modalities have increasingly been necessary to explain diverse biological processes involving cell loss. Two regulated forms of nonapoptotic cell death, necroptosis and ferroptosis, have been shown recently to play significant roles in numerous biological contexts [1,2]. While the mechanisms and physiological relevance of necroptosis have been reviewed recently [3], we focus here on the molecular mechanisms controlling ferroptosis and its relevance to health and disease.

Early life on Earth developed in the absence of oxygen [4]; approximately 2.4 billion years ago, the composition of the atmosphere changed dramatically, probably due to oxygen production from emerging photosynthetic organisms [5,6]. After this Great Oxygenation Event, oxygen in the atmosphere ultimately rose from only trace abundances to its current 21% abundance [5,6]. The emergence of copious amounts of oxygen in the atmosphere was challenging for organisms with membranes having polyunsaturated lipids that contain bis-allylic carbons, because these are highly susceptible to lipid peroxidation in the presence of oxygen [7]. This peroxidation reaction is dramatically accelerated by divalent metals, especially Fe(II) [7].

While life probably originated using saturated amphiphilic lipids (i.e., charged but hydrophobic molecules lacking carbon–carbon double bonds, such as simple fatty acids) [8], before long, monounsaturated and polyunsaturated lipids were integrated into lipid metabolism and membrane biochemistry, as they allow increased tunability of membrane fluidity [9]. However, the presence of these polyunsaturated fatty acids created a liability after the Great Oxygenation Event, especially because of the abundance of Fe(II) and Fe(II)-dependent enzymes: without a means to prevent lipid peroxidation, these membranes became a source of damaging oxidative species as reactive lipid peroxides were generated.

These observations on the early origin and essential functions of polyunsaturated fatty acids, Fe(II)-dependent oxidation chemistry and abundant oxygen suggest the hypothesis that the evolution of defenses against lipid peroxidation was an early selective event in the development

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of life. Over time, this defense system was adapted to a more complex control mechanism that allowed fine-tuned control over lipid peroxidation, enabling it to be harnessed to generate signaling molecules (e.g., in the inflammatory cascade), suppressed to preserve cell integrity under high peroxidation stress (e.g., in neurons or renal tubules), or unleashed to cause lethal lipid peroxidation (e.g., in nascent neoplastic cells).

Ferroptosis is this nonapoptotic, peroxidation-driven form of regulated cell death that requires abundant and accessible cellular iron; the existence of this ancient form of cell death was unknown and discovered only recently using a pharmacological approach [10]. The first ferroptosis-inducing compounds, erastin [11] and RSL3 [12], were discovered using high-throughput screening of small-molecule libraries [11,12]. The mode of cell death induced by these compounds was surprisingly found to be nonapoptotic, as cells treated with erastin and RSL3 died in the absence of apoptotic hallmarks [11–13], and in cells where the core apoptosis machinery – caspases, BAX, and BAK – was suppressed [14]. Although nonapoptotic, erastin-induced cell death proceeds normally on knockdown of RIPK1/RIPK3 or pharmacological inhibition of RIPK1 [10,15] (our unpublished data), a known component of necroptosis [16]. Therefore, the cell death phenotype induced by erastin and RSL3 is distinct from other reported modalities, including apoptosis and necroptosis.

Further studies [13,17] identified lipophilic antioxidants (α-tocopherol, butylated hydroxytoluene, and β-carotene) as strong suppressors of erastin-induced cell death, suggesting that ROS, probably lipophilic in nature, are involved in this cell death process. Analysis with dichlorofluorescein (DCF), a ROS-detecting dye, revealed that erastin causes the generation of ROS in sensitive cell lines [10,13]. Moreover, iron chelators were identified as inhibitors of cell death induction after RSL3 treatment, revealing the requirement for cellular iron [12].

Using modulatory profiling, an unbiased pharmacological and genetic profiling system in which lethal compounds are classified based on their functional profiles [14,18], erastin and RSL3 were found to cluster together, suggesting that they share a similar cell death mechanism. This erastin–RSL3 cluster is distinct from other lethal compounds that induce apoptosis and necrosis. Taking these findings together, the mode of cell death induced by erastin, RSL3, and related compounds was proposed to be a previously unrecognized form of cell death termed ferroptosis [ferro, 'ferrous ion' (Fe^{2+}); ptosis, 'fall'], suggesting a critical role for cellular iron in this regulated form of oxidative cell death [10].

Mechanism of Ferroptosis Induction by Erastin and RSL3

Key regulators of ferroptosis have been discovered through characterizing the mechanism of action of erastin and RSL3 using multipronged approaches (Figure 1, Key Figure).

Ferroptosis Induction by System x_c^- Inhibition

Glutamate-induced toxicity can be initiated by calcium influx after glutamate receptor activation [19] or by competitive inhibition of system x_c^-, the glutamate/cystine antiporter [20,21] (Box 1). Glutamate-induced neurotoxicity is an oxidative, iron-dependent process, suggesting that ferroptosis is involved [22,23]. Calcium chelators showed no effect on erastin-induced cell death [14], suggesting that glutamate receptor activation is not involved. In addition, the modulatory profiles of erastin and sulfasalazine (SAS), an inhibitor of system x_c^-, were similar, suggesting that erastin might act as a system x_c^- inhibitor to initiate ferroptosis [10]. Erastin treatment abolished the import of radiolabeled cystine [10], a substrate for the system x_c^- antiporter, confirming that erastin inhibits system x_c^-.

One metabolic consequence of system x_c^- inhibition is depletion of the intracellular cysteine pool, which is a precursor for glutathione synthesis. A metabolite profiling assay revealed that