**SnapShot: Ferroptosis**

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**Abstract:**

Ferroptosis is a regulated form of cell death that occurs when phospholipids with polyunsaturated fatty acids are oxidized in an iron-dependent manner. Research of recent years has uncovered complex cellular networks that induce or block lethal lipid peroxidation. This Snapshot gives a comprehensive overview of ferroptosis related pathways including cellular components as well as small molecule modulators regulating these processes.

**Text:**

Cells are the basic building blocks of living systems; hence, the mechanisms governing their division, proliferation, growth, differentiation, and death are critical for the diverse characteristics of life. Until the mid-20th century, death of cells was thought to be largely passive and uncontrolled. Recent decades have revealed that regulated cell death is ubiquitous in the development and homeostasis of virtually all multicellular organisms, and is dysregulated in a myriad of environmental and genetic diseases involving aberrations in the number and types of cells in organisms.

Ferroptosis is a specific form of regulated cell death driven by iron-dependent lipid peroxidation: ferroptosis is regulated in that it can be induced or suppressed by specific pharmacological and genetic perturbations. **Peroxidation of phospholipids, which compose the lipid bilayers that make up cellular membranes, is the key driver of ferroptotic death** (Stockwell et al., 2017). Regulation of ferroptosis involves controlling the abundance of key phospholipid substrates, *i.e.* the factors that drive their peroxidation, and those eliminating these peroxides (Figure).

The essential substrates for peroxidation during ferroptosis are **PL-PUFAs** (Figure, bottom left), due to their susceptibility to peroxidation chemistry. These PL-PUFAs are generated by enzymes such as **ACSL4** and **LPCATs** (green, Figure, lower left) that activate and incorporate the free PUFAs into phospholipids. PUFAs can be scavenged from the environment or dietary sources, or in some cases can be synthesized from the basic building block acetyl-CoA, through the action of acetyl-CoA carboxylase (ACC) (green, Figure, lower left). **Energy stress** and **AMPK** suppress ferroptosis by inhibiting ACC (red, Figure, lower left) (Lee et al., 2020).

Once PL-PUFAs are incorporated into necessary membrane environments, **iron-dependent enzymes and labile iron** use molecular oxygen (O2) to do the peroxidation reaction, thereby generating the PL-PUFA-OOH death signals (Figure, blue pathway). Iron-dependent enzymes found to drive ferroptosis include lipoxygenases and **cytochrome P450 oxidoreductase (POR)** (Zou et al., 2020). Labile iron is imported through the **transferrin receptor 1 (TfR1)**, and stored in ferritin. Ferritin can be degraded through an autophagy-like process known as ferritinophagy, which releases labile iron and facilitates the peroxidation reaction driving ferroptosis (blue, Figure, upper left). **Radiation** can also directly stimulate lipid peroxidation, and radiotherapy likely works in part through triggering ferroptosis (Lei et al., 2020; Ye et al., 2020). In contrast, **Prominin2** suppresses ferroptosis by facilitating the formation of multivesicular bodies containing ferritin-bound iron and as a consequence exporting iron out of cells (blue, Figure, upper left) (Brown et al., 2019).

There are three main pathways for eliminating peroxidized PL-PUFAs (red, Figure, center and lower right): the **GPX4-glutathione axis** (Stockwell et al., 2017), the **FSP1-CoQ10 axis** (Bersuker et al., 2019; Doll et al., 2019), and the **GCH1-BH4 axis** (Kraft et al., 2020):

* **GPX4** uses the cysteine-containing tripeptide **glutathione** to eliminate phospholipid peroxides (red, Figure, center). Glutathione itself is generated from cysteine, which can either be obtained from methionine through the transsulfuration pathway, or from extracellular cystine through **system xc-** (black, Figure, top center), which exchanges intracellular glutamate for extracellular cystine; cystine is the oxidized disulfide form of the amino acid cysteine (Stockwell et al., 2017). System xc- is a central hub for regulation of ferroptosis, as **CD8+ T-cell-derived IFN** triggers ferroptosis in cancer cells upon immunotherapy by downregulating *SLC7A11*, one of the two genes that composes system xc-. In contrast, **NRF2** upregulates *SLC7A11*, thereby protecting from ferroptotic cell death.
* **Reduced coenzyme Q10, also know as ubiquinol**, suppresses the formation of PL-PUFA-OOH. **FSP1** (formerly known as AIFM2) regenerates ubiquinol from ubiquinone (red, Figure, right), which is generated through the mevalonate pathway (Bersuker et al., 2019; Doll et al., 2019). FSP1 can be activated by **PPAR** (red, Figure, right), which is under the control of the **MDM2/MDMX** complex (green, Figure, right), independent of p53 (Venkatesh et al., 2020).
* **GCH1** generates the metabolite **tetrahydrobiopterin (BH4)**, which has a dual function in generating **reduced CoQ10 (ubiquinol)**, and **remodeling lipids** to disfavor lipid peroxidation (Kraft et al., 2020). Furthermore, **MUFAs**, when incorporated into phospholipids through the action of ACSL3, act through an unknown mechanism to suppress ferroptosis (red, Figure, lower right).

There are varieties of ferroptosis-inducing compounds, lipids and proteins (see **Ferroptosis Inducers Table**), as well as inhibitors of ferroptosis (see **Ferroptosis Inhibitors Table**). Finally, lipid peroxidation and some key players in ferroptosis regulation can be detected using a variety of dyes, assays, molecular markers and antibodies (see **Ferroptosis-Related Assays and Tools**).

Our increasing understanding of the mechanisms underlying lipid peroxidation and ferroptosis, the availability of tools to study this form of cell death, and emerging physiological functions of this mode of death promise a wealth of future advances in blocking or utilizing ferroptosis in the right disease context.

**Abbreviations:**

ACC, acetyl-CoA carboxylase; ACSL3, Acyl-CoA synthetase long-chain family member 3; ACSL4, Acyl-CoA synthetase long-chain family member 4; AMPK, AMP-activated protein kinase; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; BSO, buthionine sulfoximine; CoA, coenzyme A; Fe, iron; FSP1, ferroptosis suppressor protein 1; GCH1, GTP cyclohydrolase 1; GCS, glutamylcysteine synthetase; GLS, glutaminase; GPX4, glutathione peroxidase 4; GSH/GSSH, glutathione; GSR, glutathione S-reductase; GSS, glutathione synthetase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HNE, hydroxynonenal; IKE, imidazole ketone erastin; INF, interferon gamma; LPCAT3, lysophosphatidylcholine acyltransferase 3; LOX, lipoxigenases; MDA, malondialdehyde; MDM2, mouse double minute 2 homolog; MDMX, mouse double minute 4 homolog; MUFA, monounsaturated fatty acid; NRF2, nuclear factor E2-related factor 2; OXPHOS, oxidative phosphorylation; PPAR, peroxisome proliferator activated receptor alpha; PE, piperazine erastin; PL, phospholipid; POR, cytochrome P450 oxidoreductase; PUFA, polyunsaturated fatty acid; TBARS, Thiobarbituric acid reactive substances; TCA, tricarboxylic acid; TfR1, transferrin receptor 1; YAP, Yes-associated protein

**Acknowledgements:**

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