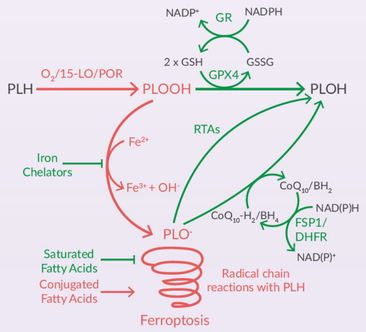
FERROPTOSIS

MECHANISMS

Induction

Polyunsaturated fatty acids (**PUFAs**) can be oxidized by cellular lipoxygenases such as **15-LO** or the cytochrome **P450 oxidoreductase (POR)** as well as non-enzymatic processes driven by the **Fenton reaction**. Their *bis*-allylic hydrogens are prone to abstraction, which leads to the production of alkyl radicals that readily react with O_2 to produce peroxyl radicals that react with other PUFAs, creating a chain reaction of lipid peroxidation that results in ferroptosis. Highly oxidizable conjugated fatty acids **(CFAs)** also elicit ferroptosis-promoting effects. Several classes of small molecules are known as ferroptosis inducers (FINs).



Suppression

Glutathione peroxidase 4 (GPX4) catalyzes the reduction of lipid peroxides at the expense of glutathione (GSH). **Ferroptosis suppressor protein 1 (FSP1)** and **dihydroorotate dehydrogenase (DHODH)** reduce coenzyme Q_{10} (CoQ_{10}) to CoQ_{10}-H_2, which halts lipid peroxidation. **GTP cyclohydrolase 1 (GCH1)** and **dihydrofolate reductase (DHFR)**, initiate the synthesis of BH_4, which protects lipids from peroxidation. **Radical-trapping antioxidants (RTAs)** terminate radical chain reactions. **Iron chelators** remove excess iron, preventing the formation of highly reactive hydroxyl radicals. Incorporation of saturated and monounsaturated fatty acids **(SFAs & MUFAs)** into cellular membranes counteracts the effects of PUFAs and CFAs.

PATHOPHYSIOLOGY

Inhibit Ferroptosis to Prevent Tissue Injury

- I/R Injury
- Transplantation
- Neurodegeneration
- Stroke
- TBI
- Fibrosis
- I/R Injury
- AKI
- I/R Injury
- Transplantation
- Islet Function (Type I Diabetes)

Promote Ferroptosis to Prevent Tumor Growth

- Gastric Cancer
- Colorectal Cancer
- Lung Cancer
- Hepatocellular Carcinoma
- Clear Cell Renal Cell Carcinoma
- Adrenocortical Carcinoma
- Pancreatic Cancer

Special thanks to Dr. Sebastian Doll from the Helmholtz Zentrum München for consultation on this diagram.

HALLMARKS

Morphological Traits

- Cell swelling/rounding
- Organelle condensation in perinuclear compartment
- Intact nucleus (no chromatin condensation)
- Reduced mitochondrial volume
- Increased mitochondrial membrane density
- Disappearance of mitochondrial cristae
- Plasma membrane rupture and membrane blowouts

Biochemical Traits

- Accumulation of redox-active metal ions (labile iron pool)
- Depletion of endogenous antioxidants (*i.e.*, GSH)
- Redox imbalance (oxidation > reduction)
- Radical chain reactions of PUFAs increase phospholipid peroxidation
- Depletion of intracellular NAD(P)H
- ATP levels remain intact

Protein Changes

- Activation of antioxidant defense *via* Nrf2 signaling
- Degradation of ferritin and GPX4
- Upregulation of ACSL4 and TfR1

METABOLIC REQUIREMENTS *IN VITRO*

- Active proliferation (withdrawal of Gln, Lys, Arg, Met, or Val leads to cell cycle arrest)
- ROS production
- Incorporation of PUFAs into phospholipids
- Accumulation of redox active metal ions (Fe^{2+}, Zn^{2+})

EXPERIMENTAL DESIGN CONSIDERATIONS

In Vitro	*In Vivo*
High oxygen partial pressure enables oxidizing cellular conditions, making the cystine/glutamate exchange system highly relevant.	Low oxygen partial pressure and varying extracellular redox conditions demotes the role for cystine exchange in favor of neutral amino acid transporters.
Serum content (*e.g.*, amino acids, lipids, iron, antioxidants, selenium) influences the ferroptotic response.	Nutrition/environmental factors that change levels of amino acids, lipids, iron, antioxidants, or selenium influence the ferroptotic response.
Ferroptosis inducers are highly efficient, causing synchronized cell death.	Ferroptosis sensitivity is restricted to certain cell types/tissues.
Under low density and limited cell-cell contact, YAP/TAZ enables ferroptosis. Confluent cells are more resistant.	Genetic deletion of GPX4 is the most reliable means to induce ferroptosis. RSL3 is not suitable *in vivo*. Iron-based nanoparticles and 2nd generation Class I FINs are in development.

Read more about the elements that induce and suppress ferroptosis at **www.caymanchem.com/ferroptosis**