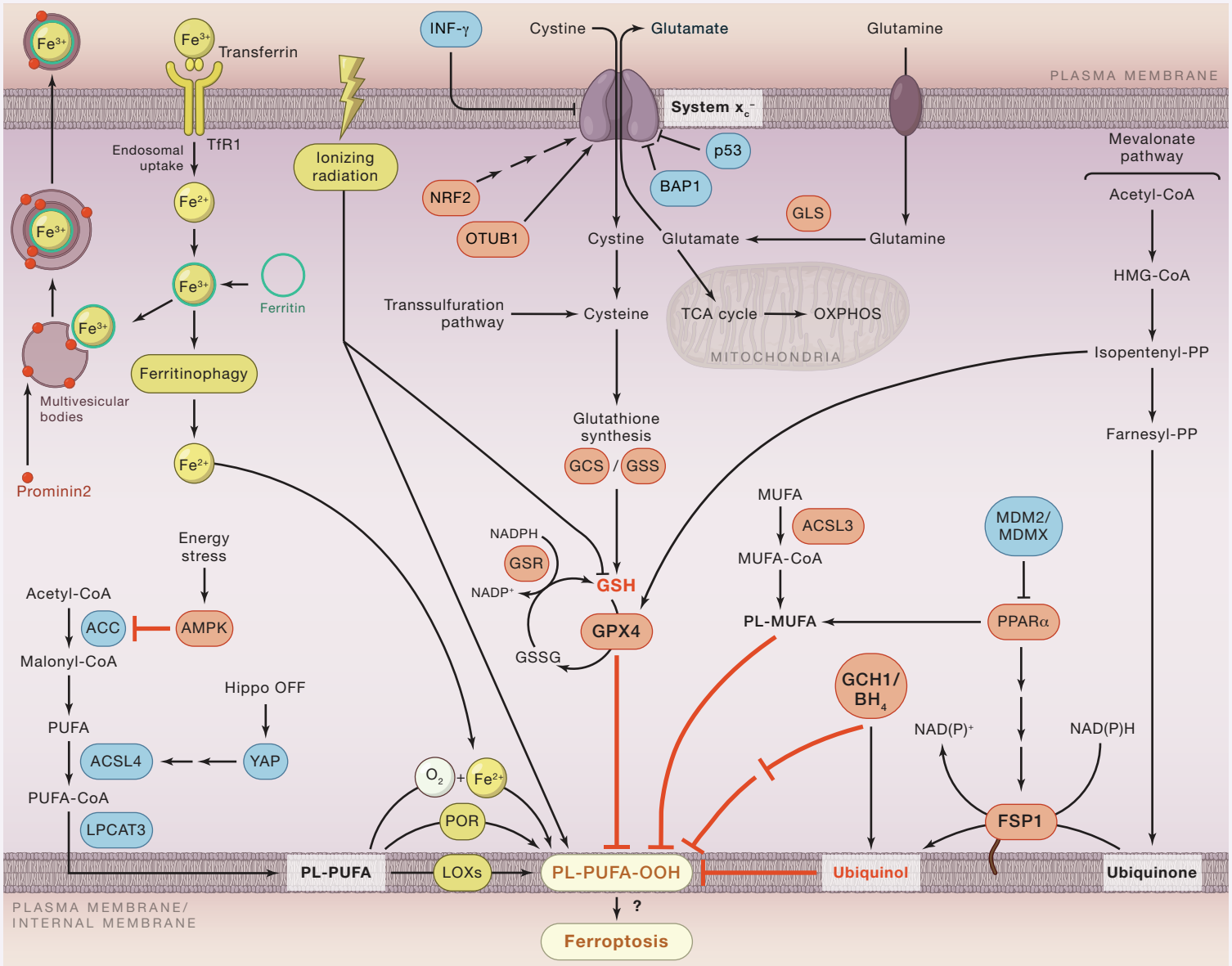
SnapShot: Ferroptosis

Kamyar Hadian[1] and Brent R. Stockwell[2,3]
[1]Assay Development and Screening Platform, Helmholtz Zentrum München, Neuherberg, Germany
[2]Department of Biological Sciences, Columbia University, New York, NY 10027, USA
[3]Department of Chemistry, Columbia University, New York, NY 10027, USA

Cell



Ferroptosis inducers	
Inducers	**Mode of action**
Erastin, PE, IKE, erastin analogs, sulfasalazine, glutamate, sorafenib	Inhibit system x_c^- → GSH depletion
INF-γ	Downregulates expression of system x_c^-
(1S,3R)-RSL3, ML162, DPI compounds	Inhibit GPX4 → lipid peroxidation
Cystine/cysteine depriviation, buthionine sulfoximine (BSO)	Inhibit GCS → GSH depletion
FIN56	Depletes CoQ_{10}; decreases GPX4 levels
$FINO_2$	Induces lipid peroxidation; indirectly inhibits GPX4
Statins	Block CoQ_{10} synthesis by mevalonate pathway inhib.
Phospholipids with two PUFA tails	Induce lipid peroxidation

Ferroptosis inhibitors	
Inhibitors	**Mode of action**
Ferrostatins, liproxstatins	Block lipid peroxidation
Vitamin E, alpha-tocopherol, trolox	Block propagation of lipid peroxidation
CoQ_{10}, idebenone	Block lipid peroxidation
Deferoxamine (DFO), ciclopirox, deferiprone	Deplete iron
Dihydrobiopterin (BH_2), tetrahydrobiopterin (BH_4)	Antioxidant effect; lipid remodeling
Deuterated PUFAs (D-PUFAs), MUFAs	Block lipid peroxidation
Lipoxygenase inhibitors (e.g. CDC, baicalein and zileuton)	Block lipid peroxidation induced by lipoxygenases

Ferroptosis-related assays and tools	
Assays and tools	**Reagent method**
BODIPY 581/591 C11	Sensor for lipid ROS levels
LiperFluo	Fluorescent lipid peroxide reagent
TBARS assay	Detects MDA product of lipid peroxidation
FENIX (fluorescence-enabled inhibited autoxidation)	Microplate-based assay to detect phospholipid peroxidation
Metabolomics/lipidomics	Detects metabolites and lipids
GSH/GSSG ratio detection kit	Quantifies glutathione levels
Fe^{2+}/Fe^{3+} quantification kit	Measures ferrous and ferric iron
Anti-TfR1(3B8 2A1 and 3F3-FMA)	Detect TfR1, a ferroptosis marker
Anti-MDA adduct (1F83)	Detects malondialdehyde
Anti-4-HNE (ab46545)	Detects 4-hydroxynonenal

See online version for legends and references

SnapShot: Ferroptosis

Kamyar Hadian[1] and Brent R. Stockwell[2,3]
[1]Assay Development and Screening Platform, Helmholtz Zentrum München, Neuherberg, Germany
[2]Department of Biological Sciences, Columbia University, New York, NY 10027, USA
[3]Department of Chemistry, Columbia University, New York, NY 10027, USA

Cells are the basic building blocks of living systems; the mechanisms governing their division, differentiation, and death are critical for life. Until the mid-20th century, the death of cells was thought to be largely 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 environmental and genetic diseases.

Ferroptosis is a form of regulated cell death driven by iron-dependent lipid peroxidation: ferroptosis 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, the factors that drive their peroxidation, and the factors that eliminate these lipid peroxides.

The substrates for peroxidation during ferroptosis are phospholipids with polyunsaturated acyl tails (PL-PUFAs) (bottom left) because of their intrinsic susceptibility to peroxidation chemistry. These PL-PUFAs are generated by enzymes such as ACSL4 and LPCATs (blue, bottom left) that activate and incorporate free PUFAs into phospholipids. PUFAs can be scavenged from the environment and dietary sources and can be synthesized from the basic building block acetyl CoA, through the action of acetyl CoA carboxylase (ACC) (blue, bottom left). Energy stress and AMPK suppress ferroptosis by inhibiting ACC (orange, bottom left) (Lee et al., 2020).

Once PL-PUFAs are incorporated into membrane environments, iron-dependent enzymes and labile iron use molecular oxygen (O_2) to do a peroxidation reaction, generating PL-PUFA-OOH (yellow 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 (yellow, top 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 (yellow, top left) (Brown et al., 2019).

There are three pathways for eliminating peroxidized PL-PUFAs (orange, middle and bottom right): the GPX4-glutathione axis (Stockwell et al., 2017), the FSP1-CoQ_{10} axis (Bersuker et al., 2019; Doll et al., 2019), and the GCH1-BH_4 axis (Kraft et al., 2020):

- GPX4 uses the cysteine-containing tripeptide glutathione to eliminate phospholipid peroxides (orange, middle). Glutathione itself is generated from cysteine, which can either be obtained from methionine through the transsulfuration pathway or from extracellular cystine through system x_c^- (purple, top middle), which exchanges intracellular glutamate for extracellular cystine; cystine is the oxidized disulfide of the amino acid cysteine (Stockwell et al., 2017). System x_c^- is a central hub for regulation of ferroptosis, as $CD8^+$ T cell-derived interferon-γ (IFN-γ) triggers ferroptosis in cancer cells upon immunotherapy by downregulating *SLC7A11*, one of the two genes that composes system x_c^-. In contrast, NRF2 upregulates *SLC7A11*, thereby protecting from ferroptotic cell death.
- Reduced coenzyme Q_{10}, also known as ubiquinol, suppresses the formation of PL-PUFA-OOHs. FSP1 (formerly known as AIFM2) regenerates ubiquinol from ubiquinone (orange, bottom right), which is generated through the mevalonate pathway (Bersuker et al., 2019; Doll et al., 2019). FSP1 can be activated by PPARα (orange, right), which is under the control of the MDM2/MDMX complex (blue, right), independent of p53 (Venkatesh et al., 2020).
- GCH1 generates the metabolite tetrahydrobiopterin (BH_4), which has a dual function in generating reduced CoQ_{10} (ubiquinol) and remodeling lipids to disfavor lipid peroxidation (Kraft et al., 2020). Furthermore, monounsaturated fatty acids (MUFAs), when incorporated into phospholipids through the action of ACSL3, act through an unknown mechanism to suppress ferroptosis (orange, bottom right).

There are several ferroptosis-inducing compounds, lipids, and proteins (see Ferroptosis Inducers Table), as well as inhibitors of ferroptosis (see Ferroptosis Inhibitors Table). Lipid peroxidation and key ferroptosis regulators can be detected using dyes, assays, molecular markers, and antibodies (see Ferroptosis-Related Assays and Tools).

Our increasing understanding of the mechanisms underlying the connections between metabolism, lipid peroxidation, and ferroptosis, the availability of tools to study this form of cell death, and its emerging physiological functions promise a wealth of future advances in exploiting ferroptosis for the understanding and treatment of disease.

ACKNOWLEDGMENTS

B.R.S. is supported by NCI grants P01CA87497 and R35CA209896 and NINDS grant R61NS109407.

DECLARATION OF INTERESTS

B.R.S. is an inventor on patents and patent applications involving ferroptosis and co-founded and serves as a consultant to Inzen Therapeutics and Nevrox.

ABBREVIATIONS

ACC, acetyl coenzyme A carboxylase; ACSL3, acyl coenzyme A synthetase long-chain family member 3; ACSL4, acyl coenzyme A synthetase long-chain family member 4; AMPK, AMP-activated protein kinase; BH_2, dihydrobiopterin; BH_4, 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/GSSG, glutathione; GSR, glutathione S-reductase; GSS, glutathione synthetase; HMG CoA, 3-hydroxy-3-methylglutaryl CoA; HNE, hydroxynonenal; IKE, imidazole ketone erastin; INF - γ, interferon gamma; LPCAT3, lysophosphatidylcholine acyltransferase 3; LOX, lipoxygenase; MDA, malondialdehyde; MDM2, mouse double minute 2; MDMX, mouse double minute 4; MUFA, monounsaturated fatty acid; NRF2, nuclear factor E2-related factor 2; OXPHOS, oxidative phosphorylation; PPARα, 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

REFERENCES

Bersuker, K., Hendricks, J.M., Li, Z., Magtanong, L., Ford, B., Tang, P.H., Roberts, M.A., Tong, B., Maimone, T.J., Zoncu, R., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature *575*, 688–692.

Brown, C.W., Amante, J.J., Chhoy, P., Elaimy, A.L., Liu, H., Zhu, L.J., Baer, C.E., Dixon, S.J., and Mercurio, A.M. (2019). Prominin2 Drives Ferroptosis Resistance by Stimulating Iron Export. Dev Cell. *51*, 575–586.

Doll, S., Freitas, F.P., Shah, R., Aldrovandi, M., da Silva, M.C., Ingold, I., Grocin, A.G., Xavier da Silva, T.N., Panzilius, E., Scheel, C.H., et al. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. Nature *575*, 693–698.

Kraft, V.A.N., Bezjian, C.T., Pfeiffer, S., Ringelstetter, L., Müller, C., Zandkarimi, F., Merl-Pham, J., Bao, X., Anastasov, N., Kössl, J., et al. (2020). GTP Cyclohydrolase 1/Tetrahydrobiopterin Counteract Ferroptosis through Lipid Remodeling. ACS Cent. Sci. *6*, 41–53.

Lee, H., Zandkarimi, F., Zhang, Y., Meena, J.K., Kim, J., Zhuang, L., Tyagi, S., Ma, L., Westbrook, T.F., Steinberg, G.R., et al. (2020). Energy-stress-mediated AMPK activation inhibits ferroptosis. Nat. Cell Biol. *22*, 225–234.

Lei, G., Zhang, Y., Koppula, P., Liu, X., Zhang, J., Lin, S.H., Ajani, J.A., Xiao, Q., Liao, Z., Wang, H., and Gan, B. (2020). The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression. Cell Res. *30*, 146–162.

Stockwell, B.R., Friedmann Angeli, J.P., Bayir, H., Bush, A.I., Conrad, M., Dixon, S.J., Fulda, S., Gascón, S., Hatzios, S.K., Kagan, V.E., et al. (2017). Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell *171*, 273–285.

Venkatesh, D., O'Brien, N.A., Zandkarimi, F., Tong, D.R., Stokes, M.E., Dunn, D.E., Kengmana, E.S., Aron, A.T., Klein, A.M., Csuka, J.M., et al. (2020). MDM2 and MDMX promote ferroptosis by PPARα-mediated lipid remodeling. Genes Dev. Published online February 20, 2020. 10.1101/gad.334219.119.

Ye, L.F., Chaudhary, K.R., Zandkarimi, F., Harken, A.D., Kinslow, C.J., Upadhyayula, P.S., Dovas, A., Higgins, D.M., Tan, H., Zhang, Y., et al. (2020). Radiation-Induced Lipid Peroxidation Triggers Ferroptosis and Synergizes with Ferroptosis Inducers. ACS Chem. Biol. *15*, 469–484.

Zou, Y., Li, H., Graham, E.T., Deik, A.A., Eaton, J.K., Wang, W., Sandoval-Gomez, G., Clish, C.B., Doench, J.G., and Schreiber, S.L. (2020). Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. Nat. Chem. Biol. *16*, 302–309.

  DOI https://doi.org/10.1016/j.cell.2020.04.039