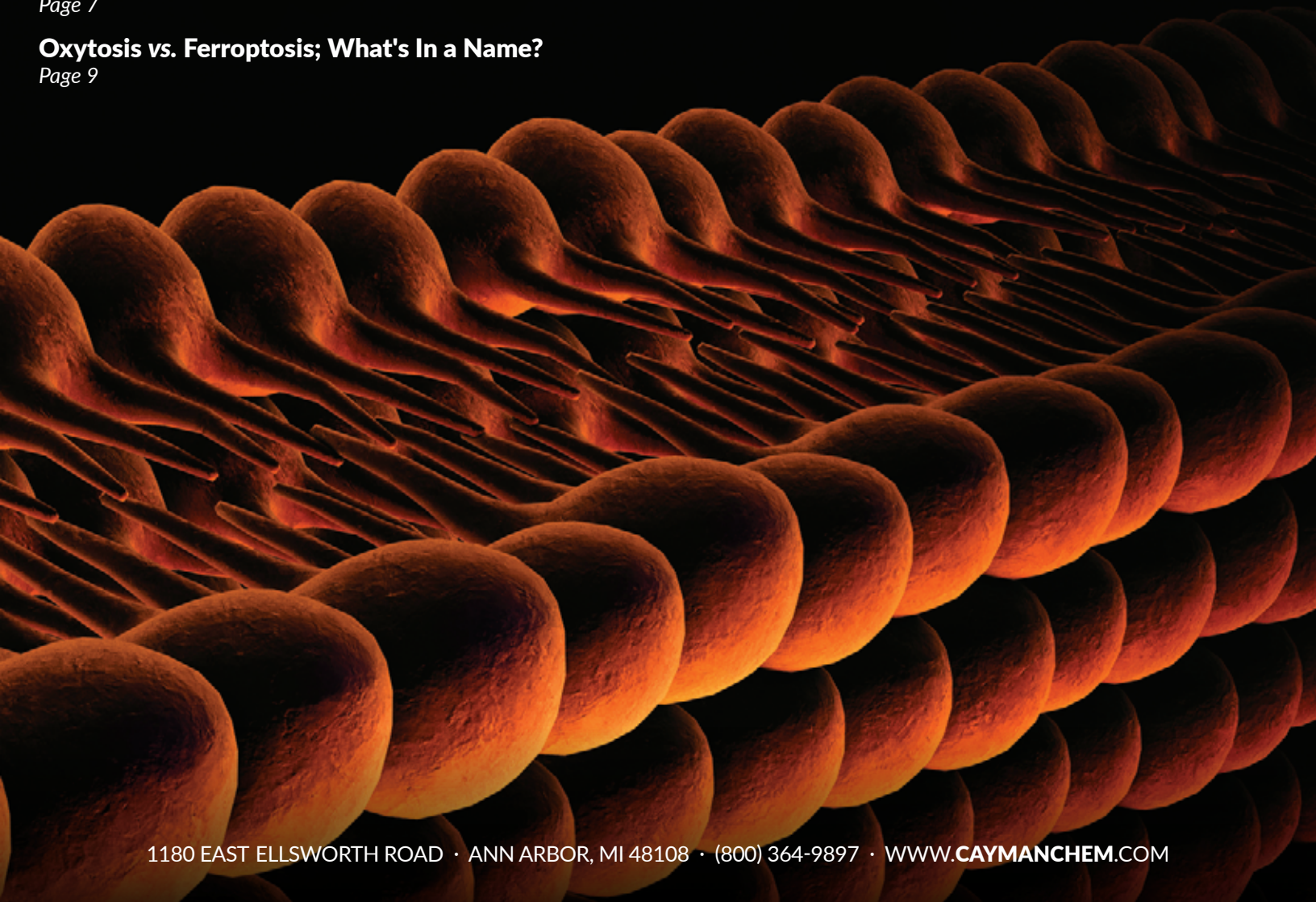
CAYMANCURRENTS
ISSUE 30 | FALL 2018

Cayman CHEMICAL

FERROPTOSIS: A New Form of Cell Death



1180 EAST ELLSWORTH ROAD · ANN ARBOR, MI 48108 · (800) 364-9897 · WWW.CAYMANCHEM.COM

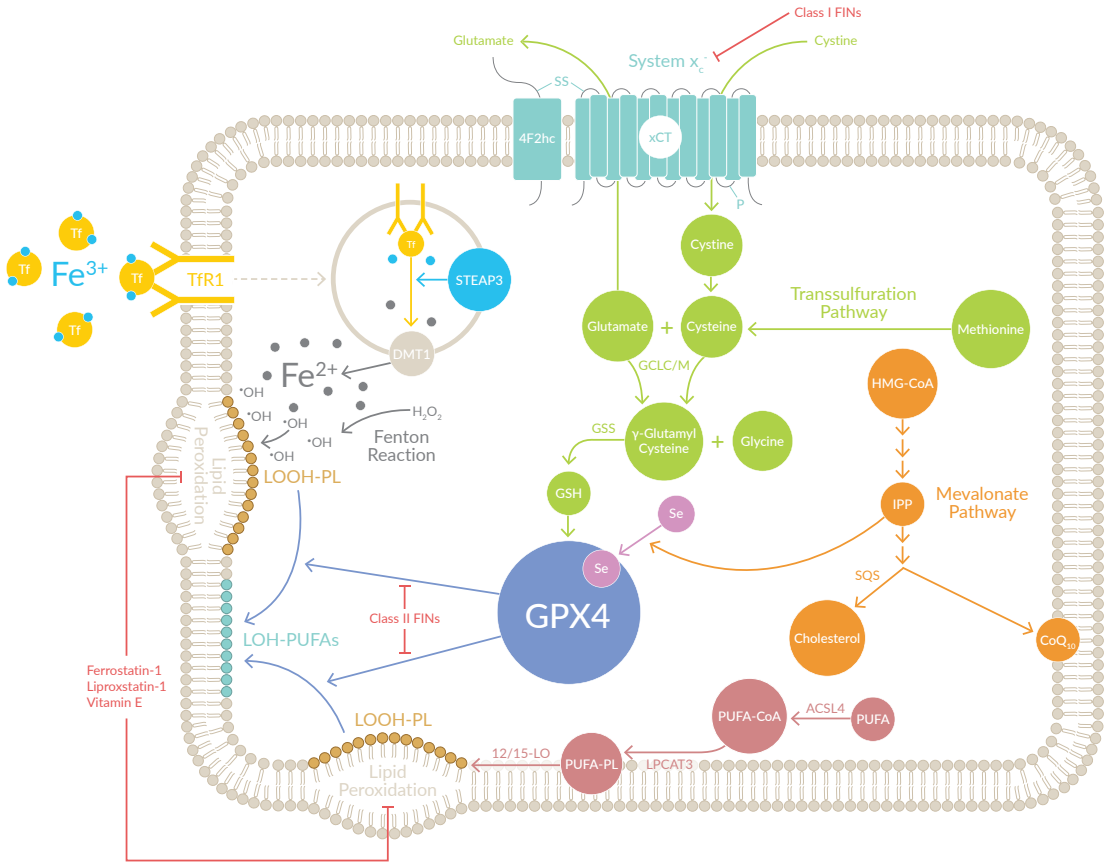
The Ferroptosis Pathway: Structure, Function, and Modulation

Leslie Magtanong and Scott J. Dixon

Department of Biology, Stanford University

Cell death is a critical process required for normal development (*e.g.*, removal of interdigital webs) and the maintenance of proper homeostasis throughout an organism's lifetime (*e.g.*, immune cell turnover). Cell death can be executed *via* caspase-mediated apoptosis or through one of a growing number of non-apoptotic cell death processes, including necroptosis, pyroptosis, parthanatos, and others.[1] Ferroptosis is one of the more recently described forms of non-apoptotic cell death.[2] This is an oxidative, iron-dependent process with cell death resulting from the accumulation of lipid reactive oxygen species (ROS) to toxic levels.[3,4] Unlike apoptosis and other forms of non-apoptotic cell death, there is no evidence to date that the execution of ferroptosis requires one specific effector protein (*e.g.*, a pore-forming protein). Rather, this lethal process is centered on the iron-dependent, oxidative destruction of membrane lipids.

As implied in its name, ferroptotic cell death is defined by the requirement for iron. Extracellular iron is bound by the protein transferrin, and internalized by the transferrin receptor.[5,6] Iron is released from internalized transferrin/transferrin receptor complexes from the lysosome into the cytoplasm.[5,6] Within the cytosol, free iron can participate in Fenton chemistry to generate hydroxyl radicals. These radicals can then react with polyunsaturated acyl chains found in membrane phospholipids to generate lipid radicals that are easily attacked by oxygen to create highly reactive lipid peroxyl radicals. In turn, these radicals can abstract protons from neighboring phospholipids, leading to the generation of additional lipid peroxides and the propagation of damage throughout the membrane. This process of autoxidation may be sufficient to cause frank membrane permeabilization during ferroptosis in some cells,[7] while in others it is likely that the enzymatic oxidation of PUFAs by iron-containing 12/15-lipoxygenase (LO; *i.e.*, LOX) enzymes also contributes to this process.[8-10]



The ferroptosis pathway. Cystine import *via* system x_c^- is used in the synthesis of reduced glutathione (GSH). GSH is a cofactor for the selenoenzyme GPX4. GPX4 is a lipid hydroperoxidase that reduces potentially toxic lipid peroxides to lipid alcohols. When cells are depleted of cystine (*e.g.*, by Class I FINs), or when GPX4 is inhibited directly (*i.e.*, by Class II FINs), lipid peroxides accumulate and lipid reactive oxygen (ROS) species are formed, which can lead to membrane permeabilization and ferroptotic cell death. Lipid ROS accumulation and ferroptosis can be attenuated by small molecule antioxidants including ferrostatin-1 and liproxstatin-1. Lipid oxidation requires both the incorporation of polyunsaturated fatty acids (PUFAs) into membrane phospholipids (PLs) as well as the import of iron into the cell *via* the transferrin-transferrin receptor pathway, and release of internalized iron from the lysosome.

Normally, highly reactive lipid peroxides are reduced to non-reactive lipid alcohols by the activity of the essential enzyme glutathione hydroperoxidase 4 (GPX4). GPX4 is a selenocysteine-containing enzyme that requires the reduced form of the antioxidant tripeptide glutathione (GSH) for its activity.[11] Cysteine is the rate-limiting substrate in GSH biosynthesis, an ATP-dependent process wherein cysteine is conjugated to glutamate and glycine. Cysteine can be synthesized from methionine (*via* transsulfuration) but can also be imported into the cell as cystine (Cys2, the oxidized form of cysteine) through a sodium-independent antiporter called system x_c^-. System x_c^- is a heterodimer composed of xCT (encoded by *SLC7A11*), a heavy chain, and 4F2 (encoded by *SLC3A2*), a light chain.[12] This antiporter exchanges intracellular glutamate for extracellular Cys2 in a one-to-one ratio. Once in the cytosol, cystine is reduced to cysteine, which enters the glutathione biosynthesis pathway. By maintaining the intracellular pool of cysteine, system x_c^- activity is a key node preventing the onset of ferroptosis in many cells. When GSH levels fall below a certain threshold, GPX4 is no longer active, lipid ROS accumulate to toxic levels and cells undergo ferroptotic cell death.[2,13]

Ferroptosis can be induced by inhibiting the system x_c^--glutathione-GPX4 axis at various points. Small molecules that deplete glutathione levels are referred to as Class I ferroptosis-inducing compounds (FINs), while those that cause ferroptosis through direct inhibition of GPX4 without depleting glutathione are termed Class II FINs.[14] The Class I FIN, erastin, was initially described to be selectively lethal to engineered tumor cells expressing oncogenic $HRAS^{V12}$.[15,16] Erastin (and analogs), the tyrosine kinase inhibitor sorafenib (*i.e.*, BAY 43-9006), and the antirheumatic drug sulfasalazine can all inhibit system x_c^-, deplete GSH, and trigger ferroptosis.[2,17] Treatment with Class II FINs such as (1S,3R)-RSL3, ML-162, and related molecules covalently bind to and inactivate GPX4.[8,14] The endoperoxide $FINO_2$ promotes ferroptosis both by decreasing GPX4 activity and oxidizing ferrous iron.[18] Finally, the oxime-containing molecule FIN56 induces ferroptosis by reducing GPX4 protein levels and also interfering with the synthesis of the endogenous lipophilic antioxidant metabolite coenzyme Q_{10} (CoQ_{10}).[19] Other molecules, like statins, may also induce ferroptosis indirectly by destabilizing GPX4 protein and inhibiting CoQ_{10} synthesis.[20] While Class I FINs have shown some promise as inducers of ferroptosis in cancer cells *in vivo*, the development of analogs with greater potency *in vivo* remains a high priority.[14,17,21] Inhibiting GPX4 has recently been pinpointed as a means to selectively kill drug-resistance cancer cell sub-populations.[20,22,23] However, to date, none of the existing Class II FINs have shown *in vivo* activity. The development of such agents will be necessary to further investigate the potential for targeting GPX4 in cancer *in vivo*. Other agents, such as iron-binding nanoparticles, may provide an alternative means to induce ferroptosis *in vivo*.[24]

Limiting the accumulation of lipid ROS prevents ferroptosis, even when GSH is depleted or GPX4 is inactivated. Given the central importance of iron and membrane polyunsaturated fatty acyls, mutations that disrupt either iron uptake or the incorporation of lipids into cellular membranes can both act as potent suppressors of ferroptosis. Thus, genetic silencing of the transferrin receptor limits iron accumulation and the induction of ferroptosis.[25,26] Incorporation of iron into iron-sulfur clusters also serves as an endogenous mechanism of limiting free iron accumulation within the cytosol, and is especially important to limit ferroptosis during hypoxia.[27] The incorporation of polyunsaturated fatty acids (PUFAs) into membrane lipids first requires the activation of the free fatty acids into PUFA-CoAs by acyl-CoA synthetase long chain family member 4 (ACSL4) and incorporation of the PUFA-CoA into lipids by lysophosphatidylcholine acyltransferase 3 (LPCAT3).[2,28-30] Genetic perturbation of these enzymes can, therefore, block cell death under ferroptosis-inducing conditions by eliminating the key substrate (*i.e.*, PUFA-containing lipids) required for the execution of this process.

Natural product and synthetic small molecule iron chelators and lipophilic antioxidants are also effective inhibitors of cell death induced by both classes of FINs.[4] Deferoxamine is a bacterial siderophore that chelates ferric iron. It was one of the first molecules found to inhibit ferroptosis.[2,25] Lipophilic antioxidants such as ferrostatin-1 and liproxstatin-1 can also all block ferroptosis *in vitro* and, with varying degrees of potency, *in vivo*.[2,13,31,32] Structurally, ferrostatin-1 and liproxstatin-1 are both diarylamines. Mechanistically, their antioxidant activity stems from their ability to trap acyl chain-carrying peroxyl radicals in lipid bilayers.[33-35] Other small molecules such as vitamin E hydroquinone may prevent ferroptosis by inhibiting the function of LO enzymes.[36] Finally, interfering with the metabolic conversion of glutamine to glutamate using compound 968 can also attenuate ferroptosis in some models, but the biochemical mechanism connecting glutamine to iron and/or lipid ROS remains poorly defined.[26] Nonetheless, these ferroptosis inhibitors have been demonstrated to prevent cellular damage and death in a wide range of *ex vivo* and *in vivo* models of acute and chronic pathological cell death,[2,13,26,32] and testing in more complex diseases models or human clinical trials should be anticipated in the future.

› Read About the Authors on Page 6
› View article references on Page 10

Ferroptosis Inducers

Class I FINs

System x_c^-

The cystine-glutamate exchange transporter regulates intracellular glutathione (GSH) content to balance the cellular redox state. Extracellular cystine is transported into the cell, which is converted into cysteine for GSH synthesis. Cells undergoing ferroptosis have very low levels of GSH and a disrupted oxidation-reduction balance. Cayman offers several ferroptosis inducers that interfere with GSH production. Antibodies and assay kits are also available to measure GSH as well as GSH reductase and transferase activity in cells.

System x_c^- Inhibitors

Item No.	Product Name	Summary
17754	Erastin	Inhibits uptake of cystine through system x_c^-
27087	Erastin2	Inhibits the system x_c^- cystine-glutamate transporter
16106	5-Octyl D-glutamate	Used to increase levels of cytoplasmic D-glutamate
10009644	Sorafenib	Inhibits cystine uptake, interfering with GSH production
15025	Sulfasalazine	A prodrug of 5-aminosalicylic acid that inhibits cystine uptake

GSH Depleters

Item No.	Product Name	Summary
10024	Acetaminophen	Decreases intracellular glutathione levels
16115	N-Acetyl-4-benzoquinone imine	Acetaminophen metabolite that conjugates with GSH during clearance; at toxic doses depletes available GSH liver reserves
14484	L-Buthionine-(S,R)-Sulfoximine	Depletes GSH by inhibiting γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis

GSH Analogs and Assay Kits

Item No.	Product Name	Summary
703002	Glutathione Assay Kit	Measures both GSH and GSSG with the use of glutathione reductase
600360	Glutathione Cell-Based Detection Kit (Blue Fluorescence)	Utilizes MCB, a highly fluorescent GSH probe, to quantify GSH levels in whole cells
14953	Glutathione ethyl ester	A cell-permeable derivative of GSH
26404	Glutathione Fluorescent Assay Kit	Utilizes an eosin-GSSG substrate to measure GSH + GSSG in cells and tissue as well as GSSG and mixed disulfides (cysteinyl-glutathione and PSSG) in plasma
10007461	L-Glutathione, reduced	Nucleophilic co-substrate to GSH transferases and electron donor to GPXs
703202	Glutathione Reductase Assay Kit	Measures GR activity by measuring the rate of NADPH oxidation

Class II FINs

GPX4

Glutathione peroxidase 4 (GPX4) catalyzes the reduction of lipid peroxides at the expense of reduced GSH. When GPX4 activity is hindered, lipid peroxides accumulate and ultimately cause cell death. Cayman offers direct suppressors of GPX4 activity that can be used to induce ferroptosis. These compounds work by covalent interaction with the selenocysteine active site of GPX4. A GPX4 antibody and GPX assay kit are also available to study this enzymatic activity.

GPX4 Inhibitors

Item No.	Product Name	Summary
23662	Altretamine	Anticancer agent that can inhibit GPX4 activity without depleting cells of GSH
20455	ML-162	Inhibits GPX4; more potent and selective than (1S,3R)-RSL3
23282	ML-210	Inhibits GPX4 to induce ferroptosis
19288	(1S,3R)-RSL3	Directly binds the selenocysteine active site of GPX4
11352	Withaferin A	Acts through a dual mechanism by either activating the Nrf2 pathway by targeting KEAP1 or inactivating GPX4

GPX Assay and GPX4 Antibody

Item No.	Product Name	Summary
703102	Glutathione Peroxidase Assay Kit	Measures GPX activity indirectly by a coupled reaction with glutathione reductase
10005258	GPX4 Polyclonal Antibody	Can be used for Western blot analysis on samples from mouse, rat, or pig

MVA Pathway

The mevalonate pathway plays a key role in GPX4 maturation, as isopentenyl pyrophosphate (IPP) contributes to the complicated insertion of selenocysteine into the catalytic center of GPX4, which is important for its antioxidant activity. This pathway is also important for the synthesis of coenzyme Q_{10}. Cayman offers a ferroptosis inducer that triggers the production of reactive oxygen species (ROS) likely through a dysregulated MVA pathway.

MVA Pathway Modulator

Item No.	Product Name	Summary
25180	FIN56	Reduces expression of GPX4 protein; also binds to and activates squalene synthase in a GPX4 degradation-independent manner

Iron Oxidation

Excessive iron metabolism contributes to ferroptosis by producing oxidative stress. Any increases in iron uptake or reduced capacity for iron storage contributes to iron overload and the potential to generate highly reactive hydroxyl radicals through the Fenton reaction. These radicals can oxidize PUFAs in lipid membranes, creating lipid hydroperoxides. Cayman offers endoperoxides that act as ferroptosis inducers by oxidizing Fe^{2+}, which promotes the production of lipid ROS.

Iron Oxidizers

Item No.	Product Name	Summary
11816	Artemisinin	Iron(II) oxide-reactive endoperoxide that generates ROS
11817	Artesunate	Iron(II) oxide-reactive endoperoxide that generates ROS
25096	(−)-$FINO_2$	Oxidizes iron, resulting in the loss of GPX4 enzymatic activity

Fluorescent Iron Indicator

Item No.	Product Name	Summary
25393	Phen Green SK diacetate	A green fluorescent heavy metal indicator

Ferroptosis Inhibitors

The presence of ferroptosis can be confirmed by looking at whether cell death is prevented by pertinent inhibitors and by measuring lipid peroxides. Cayman offers inhibitors for each of the major nodes within the ferroptotic pathway.

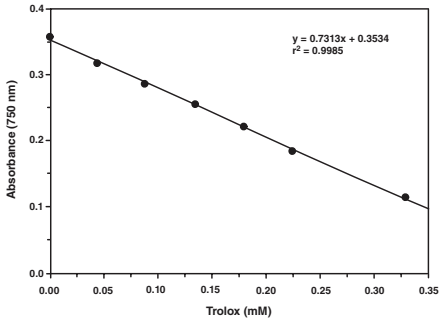
Featured Lipid Peroxide Scavengers

Item No.	Product Name	Summary
17729	Ferrostatin-1	Inhibits ferroptosis by trapping acyl chain-carrying peroxyl radicals in lipid bilayers
25492	Ferrostatin-1 Diyne	A ferroptosis inhibitor with a diyne tag for localizing ferrostatin-1 in cells
17730	Liproxstatin-1	Inhibits ferroptosis by trapping acyl chain-carrying peroxyl radicals in lipid bilayers
25689	SRS11-92	Inhibits ferroptotic cell death induced by erastin
26752	SRS16-86	Inhibits ferroptosis increasing GPX4, system x_c^- cystine-glutamate transport, and GSH
26525	UAMC-3203	More stable, readily soluble ferrostatin-1 analog

Antioxidant Assay Kit

Item No. 709001

- Measure the total antioxidant capacity of plasma, serum, urine, saliva, or cell lysates
- Assay 41 samples in duplicate
- Measure antioxidant capacity in Trolox equivalents down to 44 µM
- Plate-based colorimetric measurement (750 or 405 nm)



Additional Antioxidants

Item No.	Product Name	Summary
89910	BHT	A widely used synthetic antioxidant
16837	β-Carotene	An antioxidant, precursor of vitamin A
11506	Coenzyme Q_{10}	A cofactor in the electron-transport chain whose reduced form acts as an antioxidant
20261	N-acetyl-L-Cysteine	An antioxidant and GSH precursor
25866	N-acetyl-L-Cysteine amide	An antioxidant with enhanced blood-brain permeability over NAC
70530	Ebselen	A glutathione peroxidase mimic
15475	Idebenone	A potent lipid antioxidant that prevents the generation of free radicals
89950	Mitoquinol	A mitochondria-targeted antioxidant
14981	Probenecid	Inhibits MRP1 to prevent intracellular GSH efflux
11754	Sesamolin	A plant lignin that reduces lipid peroxidation *in vivo*
10008377	α-Tocotrienol	A vitamin E analog
10008494	γ-Tocotrienol	A vitamin E analog
10011659	Trolox	A cell-permeable vitamin E derivative with antioxidant properties

Squalene Synthase (SQS) Inhibitors

Item No.	Product Name	Summary
18113	YM-53601	An SQS inhibitor that prevents cholesterol synthesis; promotes the formation of non-sterol products in the MVA pathway
17452	Zaragozic Acid A	A reversible competitive inhibitor of SQS that reduces cholesterol synthesis

Lipoxygenase (LO) Inhibitors

Item No.	Product Name	Summary
70610	Baicalein	Inhibits 12-LO and 15-LO-1; inhibits lipid peroxidation
10804	CAY10649	A direct inhibitor of 5-LO product formation
10010468	15-Lipoxygenase Inhbitor 1	cts as an antioxidant, interfering with the redox cycle of 15-LO
16119	ML351	A selective 15-LO-1 inhibitor
18537	ML355	A selective 12-LO inhibitor
70300	Nordihydroguaiaretic Acid	A non-selective LO inhibitor
10010518	PD 146176	A selective 15-LO-1 inhibitor
10006967	Zileuton	A reversible 5-LO inhibitor

Iron Chelators

Item No.	Product Name	Summary
16021	Ciclopirox	A cell-permeable iron-chelating agent
16753	Deferasirox	An iron chelator that binds iron at a 2:1 ratio
20387	Deferiprone	An iron chelator that binds iron at a 3:1 ratio
14595	Deferoxamine (mesylate)	An iron chelator and inhibitor of prolyl hydroxylases

Thiazolidinediones: Inhibitors of the acyl-CoA synthetase essential for arachidonic acid metabolism

Item No.	Product Name
71730	Ciglitazone
17681	Darglitazone
71745	Pioglitazone
71740	Rosiglitazone
71750	Troglitazone

Statins: Inhibitors of HMG-CoA reductase, which catalyzes the rate-limiting step of the MVA pathway

Item No.	Product Name
10010334	Fluvastatin
10010338	Lovastatin
10010340	Mevastatin
10010342	Pravastatin
10010344	Simvastatin

About the Authors

LM

Leslie Magtanong, Ph.D.

Dr. Magtanong is a Research Scientist in the Department of Biology at Stanford University. Her research has focused on genetic networks and the genetic regulation of cellular phenotypes in yeast, mycobacteria, and mammalian cells. Her current studies are focused on elucidating the molecular mechanisms that regulate ferroptosis, with a particular focus on the role of lipid metabolic enzymes in this process.

SD

Scott J. Dixon, Ph.D.

Dr. Dixon is an Assistant Professor in the Department of Biology at Stanford University. As a postdoctoral fellow he helped spearhead early studies on ferroptosis, characterizing the basic features of this process and identifying key regulatory proteins and pathways. Dr. Dixon is currently interested in understanding the biochemical regulation of this pathway and the discovery of new ferroptosis inhibitors. He hopes that this work will result in new treatments for a variety of diseases where ferroptosis is thought to occur.

Lipid Peroxidation

Polyunsaturated fatty acids (PUFAs) are highly susceptible to oxidative damage. Lipoxygenases (LOs), particularly 15-LO, which normally uses free PUFAs as substrates, is capable of oxidizing arachidonic acid esterified to phosphatidylethanolamine (PE) within the phospholipid membrane and generating hydroperoxy-PEs (*e.g.*, 15-HpETE-PE). If GPX4 inadequately reduces hydroperoxy-PEs, their accumulation will serve as a lethal signal to trigger ferroptosis. Cayman scientists are experts in building the tools needed to study how oxidized lipids are involved in the ferroptotic process. This includes providing active LO enzymes as well as oxidized lipid standards that can be quantified as an index of lipid peroxidation.

Oxidized Lipid Mediators

Item No.	Product Name	Summary
34230	5(S)-HETE*	A 5-LO metabolite of arachidonic acid
34510	11(S)-HETE	A monohydroxy fatty acid produced by the non-enzymatic oxidation of arachidonic acid
34570	12(S)-HETE*	The predominant lipoxygenase product of mammalian platelets
34720	15(S)-HETE*	A major arachidonic acid metabolite from the 15-LO pathway
34001	(S)-HETE HPLC Mixture	Contains each of the (S)-monohydroxy lipoxygenase products of arachidonic acid
44230	5(S)-HpETE	A PUFA produced by the action of 5-LO on arachidonic acid
44570	12(S)-HpETE	A 12-LO metabolite of arachidonic acid
44720	15(S)-HpETE	A product of 15-LO metabolism of arachidonic acid
44001	Hydroperoxy HPLC Mixture	A combination of standards for lipid/lipoxygenase research

**deuterated internal standard and MaxSpec® quantitative standard are also available*

Oxidized Phospholipids

- Derived from enzymatic catalysis of either SAPC or SAPE by 15-LO
- Used to profile oxidized arachidonic and adrenic phospholipids navigating cells to ferroptosis

Item No.	Item Name
21138	1-Stearoyl-2-15(S)-HETE-*sn*-glycero-3-PC
21139	1-Stearoyl-2-15(S)-HETE-*sn*-glycero-3-PE
26531	1-Stearoyl-2-15(S)-HpETE-*sn*-glycero-3-PC
25856	1-Stearoyl-2-15(S)-HpETE-*sn*-glycero-3-PE

Lipoxygenases

Item No.	Product Name	Summary
60700	Lipoxygenase from Glycine max (soybean) - Purified	Contains primarily 13-lipoxygenase-1 and lipoxygenase Lx3 which are non-heme, non-sulfur ferroproteins
60402	5-Lipoxygenase (human recombinant)	Catalyzes the formation of 5(S)-HpETE from arachidonic acid
10341	12-Lipoxygenase (platelet-type, mouse recombinant)	Catalyzes the formation of 12-HpETE from arachidonic acid
10011263	15-Lipoxygenase-2 (human recombinant)	Metabolizes arachidonic acid to produce 15(S)-HETE

Lipoxygenase Antibodies

Item No.	Product Name	Summary
160402	5-Lipoxygenase Polyclonal Antibody	**Applications:** IF, IHC, WB • **Cross Reactivity:** (-) 12-LO and 15-LO
10007820	5-Lipoxygenase (Phospho-Ser^{523}) Polyclonal Antibody	**Application:** WB

Lipid ROS Detection

Several convenient methods to detect the presence of lipid ROS are available. This includes assays for direct quantification of lipid hydroperoxides as well as assays that quantify end-product reactive aldehydes, such as malondialdehyde (MDA) or 4-hydroxy nonenal (4-HNE).

Lipid ROS Assays

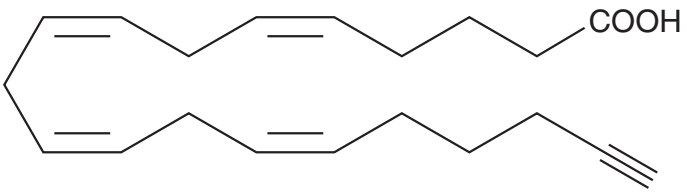
Item No.	Product Name	Summary
705002	Lipid Hydroperoxide (LPO) Assay Kit	Measures hydroperoxides directly utilizing the redox reactions with ferrous ions *(100 dtn format designed to be read using a single-tube spectrophotometer)*
705003	Lipid Hydroperoxide (LPO) Assay Kit (96 well)	Measures hydroperoxides directly utilizing the redox reactions with ferrous ions *(96-well format designed to be read using a 96-well microplate reader)*
700870	TBARS (TCA Method) Assay Kit	Measures MDA, a byproduct of lipid peroxidation *(includes TCA to accelerate condensation reaction of thiobarbituric acid with MDA)*
10009055	TBARS Assay Kit	Measures MDA, a byproduct of lipid peroxidation
501140	DHN-MA EIA Kit	Measures a metabolite of 4-HNE, a byproduct of lipid peroxidation
601520	ROS Detection Cell-Based Assay Kit (DCFDA)	Measures general ROS directly in living cells
601290	ROS Detection Cell-Based Assay Kit (DHE)	Measure superoxide and hydrogen peroxide levels in living cells

Click Chemistry Probe

Arachidonic Acid Alkyne

Item No. 10538

- Identify cellular arachidonic acid oxidation products
- Easily tag metabolites and derivatives



Lipid ROS Probes

Item No.	Product Name	Summary
62237	DPPP	A fluorescent probe for detection of hydroperoxides
13265	4-hydroxy Nonenal Alkyne	4-HNE modified for click chemistry

Lipid Peroxidation End-Products

Item No.	Product Name	Summary
10004413	4-hydroperoxy 2-Nonenal	The hydroperoxide precursor of 4-HNE
32100	4-hydroxy Nonenal*	A lipid peroxidation product used as a marker of lipid peroxidation
10185	4-oxo-2-Nonenal*	A more recently identified product of lipid peroxidation
10627	4-hydroxy Nonenal Glutathione (trifluoroacetate salt)*	A major adduct formed by the reaction of 4-HNE with GSH; prevents the formation of DNA adducts by trapping of 4-HNE
32110	4-hydroxy Nonenal Mercapturic Acid*	A urinary metabolite of 4-HNE

**deuterated internal standard is also available*

Read the Publication

CD8⁺ T cells regulate tumour ferroptosis during cancer immunotherapy

University of Michigan researchers, in collaboration with Cayman Chemical, found that increased oxidized lipids in tumor cells leads to ferroptosis and with immunotherapy is linked to cancer cell death.

Nature **569**, 270–274 (2019)

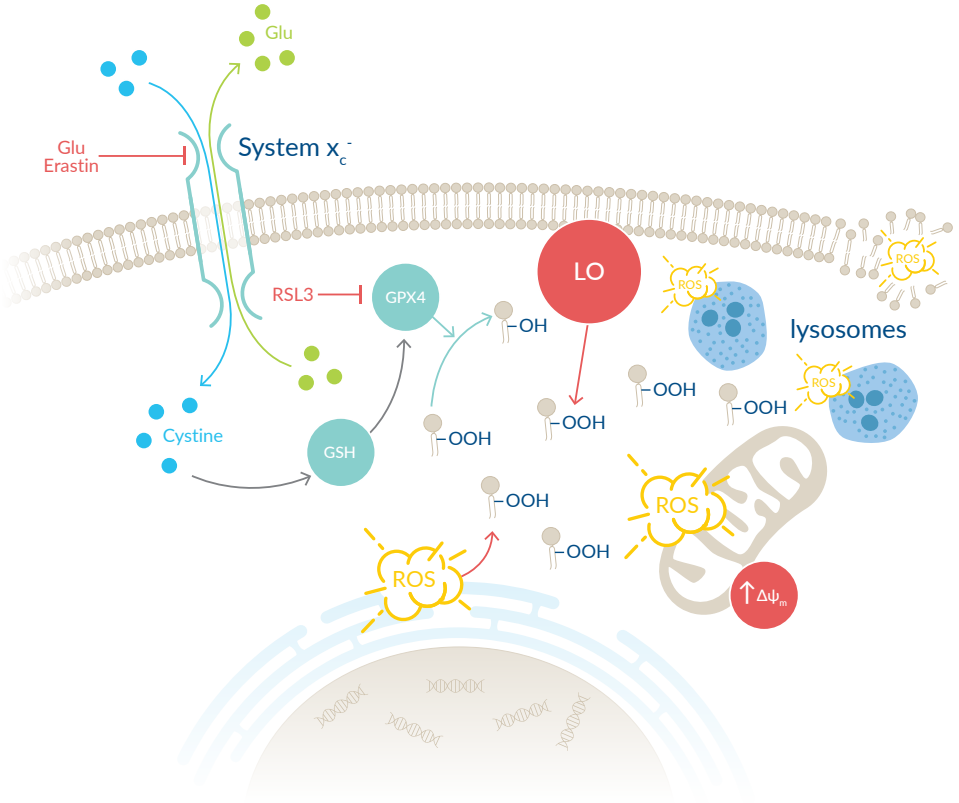
Oxytosis *vs.* Ferroptosis; What's In a Name?

Pamela Maher[§], Gamze Ates[§] and Jan Lewerenz*

[§]Salk Institute, La Jolla, CA USA; *Ulm University, Ulm, Germany

Although nerve cell death is the hallmark of many neurological diseases, the processes underlying this death are still poorly defined. However, there is a general consensus that nerve cell death predominantly proceeds by regulated processes. In 1989, Murphy and colleagues reported that glutamate could induce a calcium-dependent form of delayed cell death that was associated with depletion of intracellular glutathione (GSH) and characterized by increased oxidative stress.[1] The mechanistic link between glutamate exposure and GSH depletion proved to be the glutamate-mediated inhibition of cystine uptake *via* system x_c^-, a cystine-glutamate antiporter.[2] associated with a large increase in reactive oxygen species (ROS) generation as well as lipoxygenase (LO) activation subsequent to GSH depletion, which are followed by a final, lethal influx of calcium.[6] Oxytosis—a term that highlights both the ROS accumulation that is characteristic of this type of cell death as well as the fact that it is a form of regulated cell death distinct from apoptosis—was proposed as the name for this new form of non-apoptotic, regulated cell death.[6]

Further studies have revealed that the molecular pathways involved in the regulation of ferroptosis and oxytosis share



The common cell death pathway in oxytosis and ferroptosis. Cystine uptake by system x_c^- is inhibited by Glu and Erastin, leading to the depletion of GSH resulting in reduced GSH-dependent GPX4 activity. GPX4 can also be directly inhibited by RSL3. In the absence of GPX4 activity, ROS- or LO-derived lipid hydroperoxides (lipid icons with OOH; shown as cytosolic for illustration purposes) accumulate at various membrane sites, including mitochondria where there is a concomitant hyperpolarization of the mitochondrial membrane potential ($\Delta\psi_m$). Lysosomes also contribute to the overall ROS production. Image was adapted from Lewerenz *et al.*, 2018 with permission by CC-BY, version 4.0.

Importantly, the first reported inducer of ferroptosis, erastin,[3] was later identified to be a system x_c^- inhibitor.[4] Most of the subsequent studies addressing this delayed form of glutamate toxicity—initially called oxidative glutamate toxicity—were carried out in HT-22 cells, a hippocampal nerve cell line that was specifically selected for its sensitivity to glutamate.[5] Using this cell-based model, the biochemical events that sequentially lead to cell death were characterized in detail. It was demonstrated that this type of cell death is many similarities.[7] For example, the downstream players such as glutathione peroxidase 4 (GPX4) and LO and accumulation of mitochondria-derived ROS and nuclear translocation of apoptosis-inducing factor (AIF) are identical. In addition, transcriptomic changes in oxytosis- and ferroptosis-resistant cells correspond to identical pathways. However, some characteristics have been studied in more detail under the name of either oxytosis or ferroptosis (*e.g.*, the role of cGMP and calcium during oxytosis and the generation of lipid

peroxides during ferroptosis), and this literature can make them appear distinct.

Moreover, similar to ferroptosis, oxytosis in HT-22 cells can be inhibited by iron chelators[8,9] and exacerbated by different sources of iron.[9,10] Thus, both oxytosis and ferroptosis show the same dependency on iron, further suggesting that both pathways are highly similar. However, the whole concept of ferroptosis was recently challenged by a study demonstrating that at least in HT-22 cells, copper, the other important transition metal involved in redox metabolism in biological systems, exacerbates both cell death induced by glutamate, the prototypical insult that induces oxytosis in these cells, as well as cell death induced by the prototypical ferroptosis inducer erastin to a similar extent as iron.[10] Thus, at least under certain conditions, transition metals other than iron have the potential to exacerbate ferroptosis.

In summary, the discrepancies that have been described in the scientific literature do not indicate that ferroptosis and oxytosis are different pathways of regulated cell death but rather result from methodological differences or cell type-specific variations on a single theme. Thus, oxytosis and ferroptosis should be regarded as two names for the same cell death pathway.

References

1. Murphy, T.H., Malouf, A.T., Sastre, A., *et al. Brain Res.* **444(2)**, 325-332 (1988).
2. Bannai, S. and Kitamura, E. *J. Biol. Chem.* **255(6)**, 2372-2376 (1980).
3. Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., *et al. Cell* **149(5)**, 1060-1072 (2012).
4. Dixon, S.J., Patel, D.N., Welsch, M., *et al. Elife* **3**, e02523 (2014).
5. Davis, J.B. and Maher, P. *Brain Res.* **652(1)**, 169-173 (1994).
6. Tan, S., Schubert, D., and Maher, P. *Curr. Top. Med. Chem.* **1(6)**, 497-506 (2001).
7. Lewerenz, J., Ates, G., Methner, A., *et al. Front. Neurosci.* **12**(214) (2018).
8. Liu, Y. and Schubert, D.R. *J. Biomed. Sci.* **16:98**, 98 (2009).
9. Kang, Y., Tiziani, S., Park, G., *et al. Nature Commun.* **5**,3672 (2014).
10. Maher, P. *Free Radic. Biol. Med.* **115**, 92-104 (2018).

About the Authors

Pamela Maher, Ph.D.
Dr. Maher is a senior staff scientist at the Salk Institute with a long-standing interest in understanding how nerve cells die and what can be done to prevent it.

Gamze Ates, Pharm. D.
Dr. Ates obtained her degree in toxicology and currently works as a post-doctoral researcher in cellular neurobiology at the Salk Institute.

Jan Lewerenz
Jan Lewerenz studied medicine at the University of Hamburg and is currently the senior attending neurologist in the Department of Neurology at the University of Ulm and deputy head of the Huntington's Disease Center, Ulm.

Cover Article References

1. Galluzzi, L., Vitale, I., Aaronson, S.A., *et al. Cell Death Differ.* **25(3)**, 486-541 (2018).
2. Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., *et al. Cell* **149(5)**, 1060-1072 (2012).
3. Cao, J.Y. and Dixon, S.J. *Cell. Mol. Life Sci.* **73(11-12)**, 2195-2209 (2016).
4. Stockwell, B.R., Friedmann Angeli, J.P., Bayir, H., *et al. Cell* **171(2)**, 273-285 (2017).
5. Andrews, N.C. and Schmidt, P.J. *Annu. Rev. Physiol.* **69(1)**, 69-85 (2007).
6. Kawabata, H. *Free Radic. Biol. Med.* (2018).
7. Shah, R., Shchepinov, M.S., and Pratt, D.A. *ACS Cent. Sci.* **4(3)**, 387-396 (2018).
8. Yang, W.S., Kim, K.J., Gaschler, M.M., *et al. Proc. Natl. Acad. Sci. U.S.A.* **113(34)**, E4966-E4975 (2016).
9. Shintoku, R., Takigawa, Y., Yamada, K., *et al. Cancer Sci.* **108(11)**, 2187-2194 (2017).
10. Wenzel, S.E., Tyurina, Y.Y., Zhao, J., *et al. Cell* **171(3)**, 628-641 (2017).
11. Ingold, I., Berndt, C., Schmitt, S., *et al. Cell* **172(3)**, 409-422 (2018).
12. Lewerenz, J., Hewett, S.J., Huang, Y., *et al. Antioxid. Redox Signal.* **18(5)**, 522-555 (2013).
13. Skouta, R., Dixon, S.J., Wang, J., *et al. J. Am. Chem. Soc.* **136(12)**, 4551-4556 (2014).
14. Yang, W.S., SriRamaratnam, R., Welsch, M.E., *et al. Cell* **156(1-2)**, 317-331 (2014).
15. Dolma, S., Lessnick, S.L., Hahn, W.C., *et al. Cancer Cell* **3(3)**, 285-296 (2003).
16. Yagoda, N., von Rechenberg, M., Zaganjor, E., *et al. Nature* **447(7146)**, 864-868 (2007).
17. Dixon, S.J., Patel, D.N., Welsch, M., *et al. Elife* **3**, e02523 (2014).
18. Gaschler, M.M., Andia, A.A., Liu, H., *et al. Nat. Chem. Biol.* **14(5)**, 507-515 (2018).
19. Shimada, K., Skouta, R., Kaplan, A., *et al. Nat. Chem. Biol.* **12(7)**, 497–503 (2016).
20. Viswanathan, V.S., Ryan, M.J., Dhruv, H.D., *et al. Nature* **547(7664)**, 453-457 (2017).
21. Larraufie, M.-H., Yang, W.S., Jiang, E., *et al. Bioorg. Med. Chem. Lett.* **25(21)**, 4787-4792 (2015).
22. Tsoi, J., Robert, L., Paraiso, K., *et al. Cancer Cell* **33(5)**, 890-904 (2018).
23. Hangauer, M.J., Viswanathan, V.S., Ryan, M.J., *et al. Nature* **551(7679)**, 247-250 (2017).
24. Kim, S.E., Zhang, L., Ma, K., *et al. Nat. Nanotechnol.* **11(11)**, 977-985 (2016).
25. Yang, W.S. and Stockwell, B.R. *Chem. Biol.* **15(3)**, 234-245 (2008).
26. Gao, M., Monian, P., Quadri, N., *et al. Mol. Cell* **59(2)**, 298-308 (2015).
27. Alvarez, S.W., Sviderskiy, V.O., Terzi, E.M., *et al. Nature* **551(7682)**, 639-643 (2017).
28. Dixon, S.J., Winter, G.E., Musavi, L.S., *et al. ACS Chem. Biol.* **10(7)**, 1604-1609 (2015).
29. Kagan, V.E., Mao, G., Qu, F., *et al. Nat. Chem. Biol.* **13(1)**, 81-90 (2017).
30. Doll, S., Proneth, B., Tyurina, Y.Y., *et al. Nat. Chem. Biol.* **13(1)**, 91-98 (2017).
31. Friedmann Angeli, J.P., Schneider, M., Proneth, B., *et al. Nat. Cell Biol.* **16(12)**, 1180-1191 (2014).
32. Linkermann, A., Skouta, R., Himmerkus, N., *et al. Proc. Natl. Acad. Sci. U.S.A.* **111(47)**, 16836-16841 (2014).
33. Zilka, O., Shah, R., Li, B., *et al. ACS Cent. Sci.* **3(3)**, 232-243 (2017).
34. Shah, R., Margison, K., and Pratt, D.A. *ACS Chem. Biol.* **12(10)**, 2538-2545 (2017).
35. Poon, J.-F. and Pratt, D.A. *Acc. Chem. Res.* **51(9)**, 1996-2005 (2018).
36. Hinman, A., Holst, C.R., Latham, J.C., *et al. PLoS One* **13(8)**, e0201369 (2018).

Cayman CHEMICAL

1180 East Ellsworth Road
Ann Arbor, MI 48108
www.caymanchem.com

CONTACT US

PHONE:
(800) 364-9897 (USA and Canada only)
(734) 971-3335

FAX:
(734) 971-3640

EMAIL:
Sales: sales@caymanchem.com
Customer Service: custserv@caymanchem.com
Technical Support: techserv@caymanchem.com
Contract Services: contractresearch@caymanchem.com

SOCIAL:
www.facebook.com/caymanchemical
@CaymanChemical
www.linkedin.com/company/cayman-chemical