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Review

How do we fit ferroptosis in the family of regulated cell death?

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In the last few years many new cell death modalities have been described. To classify different types of cell death, the term 'regulated cell death' was introduced to discriminate it from 'accidental cell death'. Regulated cell death involves the activation of genetically encoded molecular machinery that couples the presence of some signal to cell death. These forms of cell death, like apoptosis, necroptosis and pyroptosis have important physiological roles in development, tissue repair, and immunity. Accidental cell death occurs in response to physical or chemical insults and occurs independently of molecular signalling pathways. Ferroptosis, an emerging and recently (re)discovered type of regulated cell death occurs through Fe(II)-dependent lipid peroxidation when the reduction capacity of a cell is insufficient. Ferroptosis is coined after the requirement for free ferrous iron. Here, we will consider the extent to which ferroptosis is similar to other regulated cell deaths and explore emerging ideas about the physiological role of ferroptosis.
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Facts

- Regulated necrosis is an expanding network of non-apoptotic cell death pathways.
- Regulated cell death involves signalling pathways that couple molecular events to cellular machinery that kills the cell.
- Apoptosis, necroptosis, and pyroptosis are initiated by high molecular weight protein platforms viz. apoptosome, necrosome or inflammasome.
- Ferroptosis occurs through Fe(II)-dependent lipid peroxidation due to an insufficient cellular reducing capacity.

Open questions

- Can ferroptosis also be induced in an active way by a high molecular weight protein platform viz. ferrosome?
- To what extent is lipid peroxidation uniquely linked to ferroptosis?
- Is ferroptosis the detrimental factor in iron-catalysed organ or systemic dysfunction?
- What is the contribution of ferroptosis to human pathologies?

'Regulated' and 'accidental' are terms used to classify different types of cell death. Regulated describes cell death that involves genetically encoded molecular machinery and a precise sequence of events starting from an extracellular or intracellular inducing signal.[1] A watershed in the field was the identification of genes required for apoptosis, the prototype form of programmed cell death. This drove cell death research that defined the sequence of cell death-eliciting events and identified an initiation phase consisting of signal transduction pathways that couple a death-inducing stimulus to the execution phase, which consists of an ensemble of biochemical activities that eventually kill the cell. This cell death research also produced important concepts about cell death processes including the central idea that cell death is not only a cell autonomous phenomenon but by intercellular communication can serve a specific biological purpose in the organism such as regulation of morphogenesis of limbs and organs,[2] inflammation by efferocytosis,[3] and tissue regeneration.[4–6] Indeed, regulated cell death can only be an evolutionary selected process when it serves the selectable benefit of the whole organism in case of multicellular organisms. This implies a prerequisite that cell death should have intercellular sensing and adaptive consequences.

Besides apoptosis other forms of regulated cell death have been drawing greater and greater attention. Foremost of these are necroptosis, pyroptosis, and ferroptosis. Although some general key features are similar in apoptosis, pyroptosis, and necroptosis, these are not discovered yet in ferroptosis. This difference was first noted by Green and Victor, who described ferroptosis as a form of cellular sabotage.[7] Cell death as a consequence of cellular sabotage would be different from an active mechanism involving a precise activation of a cytotoxic mechanism. Remember that also in case of TNF-induced necrosis, now called necroptosis, initially the mechanism was described as due to a bioenergetic crisis,[8] but now a precise

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membrane destabilizing mechanism has been discovered, that is, receptor interacting protein kinase (RIPK) 3-dependent phosphorylation of mixed lineage kinase domain-like (MLKL).[9,10] Similarly, we raise the question whether ferroptosis or oxytosis is only a consequence of insufficient cellular reduction capacity or does a pro-active mechanism exist that can initiate this mode of regulated cell death by a hypothetical ferrosome complex. Alhtough others gave already a more comprehensive description of the molecular events of ferroptosis,[11,12] we want to explore the similarities and differences between ferroptosis and the other regulated cell deaths in more detail. Evidence is emerging that ferroptosis might be an adaptive response important for the removal of cancerous or infected cells,[13] whereas excessive ferroptosis might drive degenerative diseases.[14–16]

Snapshot of the prototype modes of regulated cell death

Apoptosis, the best understood form of regulated cell death, involves the activation of cysteine proteases, caspases. Caspases are activated by a diverse set of signals generated during developmental processes, inflammatory processes and by cellular stresses and damage. During initiation these signals trigger the formation of protein complexes that activate the zymogen forms of caspases.[17] The extrinsic apoptotic pathway is typically triggered by a death-inducing signalling complex (DISC), whereas the intrinsic apoptotic pathway is initiated by the apoptosome (Figure 1a). When caspases are insufficiently activated or their activity is blocked, for, example by pharmacological or viral inhibitors, necroptosis occurs. In necroptosis, the protein complexes that induce cell death are called necrosomes. Necroptosis can be triggered by activation of death receptors, T-cell receptor, Toll-like receptors, and by viral infection.[18] The activation of RIPK3 within the necrosome typically occurs by the three RHIM-containing proteins RIPK1, Toll/IL-1 receptor domain-containing adaptor inducing IFN-β, and DNA-dependent activator of interferon regulatory factors (Figure 1b). During necroptosis, activated RIPK3 phosphorylates MLKL and forms larger complexes through the self-aggregating propensity of its RHIM domains.[19,20] MLKL can kill cells through its ability to increase the permeability of the plasma membrane (Figure 1b).[19,21–24] Moreover, recent studies revealed that during homeostasis MLKL is part of an endosomal trafficking system, whereas the execution function of MLKL is negatively controlled by the ESCRT-III membrane repair system resulting in enhanced cytokine and chemokine production.[25,26] Interestingly, a phylogenetic analysis reveals that the RIPK1/RIPK3/MLKL necroptotic axis, except for RIPK1, is poorly conserved in the animal kingdom, questioning the universal role of necroptosis during innate immunity in the animal kingdom and suggesting that other cell death modalities may functionally compensate its absence.[23,27] Similar to the involvement of the apoptosome and necrosome in, respectively, apoptosis and necroptosis, the high molecular weight inflammasome complex is crucial for the induction of pyroptosis (Figure 1c). A range of exogenous and endogenous pro-inflammatory signals can trigger formation of the inflammasome and activation of caspase-1.[28–31] Active caspase-1 regulates cytokine production but can also cause gasdermin-D cleavage, an event that triggers cell death

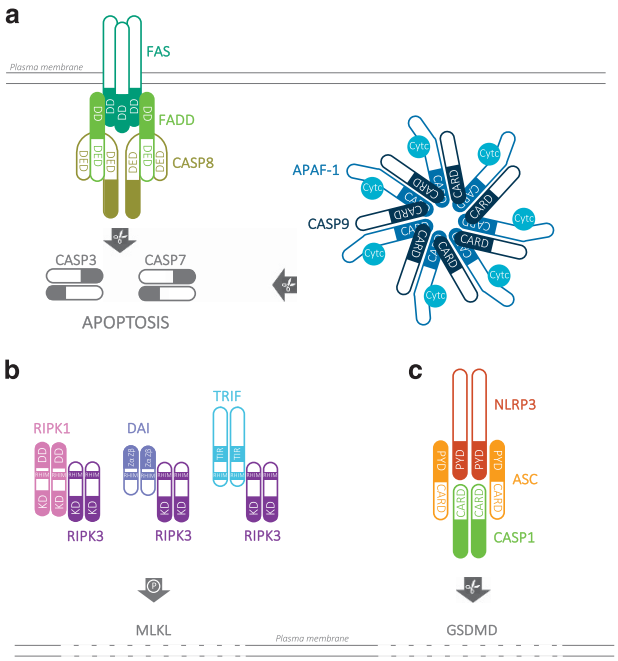

Figure 1 High molecular weight protein complexes that initiate regulated cell deaths. (**a**) Apoptosis can involve Fas receptor (FasR), FADD, and caspase-8 (Casp8) in death receptor-mediated apoptosis or Apaf-1, caspase-9 (Casp9), and cytochrome c (Cyt c) in the mitochondrial (intrinsic) pathway. (**b**) Necroptosis induced by TNF-α involves RIPK1 and RIPK3, necroptosis induced by viral DNA involves DAI/ZBP-1 and RIPK3, and necroptosis induced by dsRNA involves TRIF and RIPK3. (**c**) Pyroptosis can involve NLRP3, caspase-1 (Casp1) and ASC. Domains required for the homotypic interactions are death domains (DD), death effector domains (DED), caspase activation and recruitment domains (CARD), pyrin domains (PYD), and the RIP homotypic interaction motif (RHIM). The stoichiometry of the complexes is not shown; Apaf-1 forms a seven-membered wheel-like structure; NLRP3 may initiate the formation of large filaments containing many ASC proteins. The RIPK interactins are less well understood, by dimers of RIPK1 induce RIPK3 dimers (and higher order structures) that kill cells. For clarity upstream activating events are omitted

through pore formation in the plasma membrane.[32–35] Other inflammatory caspases activated by inflammasomes, like caspase-4/5 and -11, can also cleave gasdermin-D, which means that pyroptosis is likely relevant to other cell types as well.[36] Thus, a common feature in these prototype forms of cell death is the formation of high molecular weight complexes that are involved in the induction of a cell death execution process.

Regulated cell death fulfils a biological function

The terms ‘programmed’ or ‘regulated’ imply that these processes occur within a (patho) physiological context and fulfil biological functions. When this regulated cell death criterion is applied it is obvious that apoptosis has a range of biological roles, contributing to proper development, and tissue homeostasis, regulation of the immune system as well as a defence against cancer and various pathogens. Viruses encode a several different types of caspase-8 inhibitor,[37–41] suggesting that prevention of caspase-8-dependent apoptosis provides an advantage to the viruses. In these situations, necroptosis appears to be as a back-up cell death process to limit viral replication.[42] The importance of necroptosis as an anti-viral defence is illustrated by the existence of viral

mechanisms that in their turn subvert necroptotic cell death such as RHIM domain-containing proteins in CMV and HSV.[43–45] Pyroptosis is part of the defence against viruses and other pathogens. Cells infected with intracellular pathogens die by pyroptosis, destroying the niche the pathogen uses for replication.[46] Again, the importance of this pyroptosis is shown by pathogens evolving strategies to block this death process.[47,48] Interestingly, some pathogens appear to have co-opted this defence mechanism and use cell lysis following pyroptosis to liberate their progeny to infect new cells,[49] which may be understood in the context of the Red Queen Hypothesis. Obviously, pyroptosis not only through the processing and release of IL-1β and IL18, but also necroptosis through the production of cytokines and chemokines, in combination with damage-associated molecular patterns, can elicit inflammation.[50]

Ferroptosis, a new prototype of regulated necrosis?

The term 'Ferroptosis' was first used to describe iron-dependent cell death induced by a small molecule, erastin,[51] and has recently been extensively reviewed.[11,12,13,52] Erastin inhibits System xc$^-$, consisting of SLC3A2 and SLC7A11, which forms a glutamate/cysteine antiporter in the cell's plasma membrane that takes up cystine. Cystine is subsequently converted to cysteine, which is required for the synthesis of glutathione. Thus, erastin causes a fall in glutathione levels and increases the vulnerability of cells to oxidative damage. Ferroptosis can also be triggered by loss of Glutathione peroxidase 4 (GPX4),[15,53] an enzyme that can reduce hydrogen peroxide, organic hydroperoxides, and lipid peroxide. In the absence of GPX4, uncontrolled lipid peroxidation occurs through the accumulation of lipid radicals, lipid peroxy radicals and then Fenton-mediated lipid alkoxyl radicals. Lipid peroxidation alters the chemistry of lipids, reducing their ability to form functional cellular membranes and so can result in loss of membrane integrity and cell death.[54] Fragmentation of lipid alkoxyl radicals also produces reactive aldehydes such as malondialdehyde and 4-hydroxynonenal (4-HNE).[55] These aldehydes are reactive, but less so than free radicals, meaning they can diffuse from the site of lipid peroxidation to carbonylate proteins and so alter protein function.[56] At low levels, protein carbonylation not only may be part of signal transduction,[57] including signalling that activates apoptosis,[58] but at high levels of protein carbonylation abolish protein function and induces cell death.[59] At this time there is no direct evidence that ferroptosis involves or requires the production of reactive aldehydes, although cells resistant to ferroptosis can express high levels of enzymes that detoxify 4-HNE.[60] Two major ways to induce ferroptosis by interfering in the redox protective systems have been reported: type I inhibitors such as erastin, glutamate, sorafenib and sulfasalazine block System Xc$^-$, resulting in a drop of glutathione levels, whereas type II inhibitors such as RSL3, altretamine, ML162 and FIN56 affect GPX4, either by directly inhibiting the enzyme or by reducing levels of the protein (Figure 2).[12]

The importance of lipid peroxidation to ferroptosis is suggested by multiple lines of evidence (1) iron-chelators that inhibit iron-catalysed Fenton reactions protect against

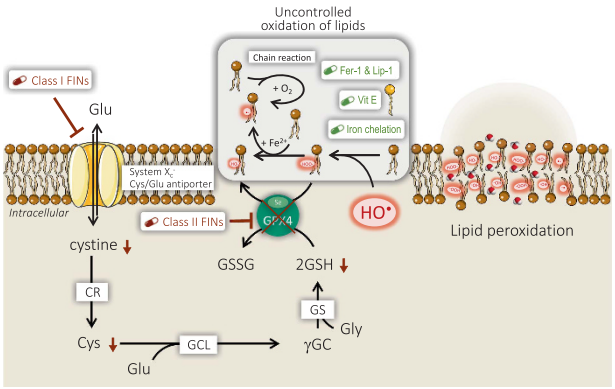

Figure 2 Induction of ferroptosis, mechanism. Type I inducers of ferroptosis inhibit System Xc(−), consisting of SLC3A2 and SLC7A11, a glutamate/cystine antiporter. Cystine is subsequently reduced to cysteine which is required for the synthesis of glutathione. Type II inducers of ferroptosis block glutathione peroxidase 4 (GPX4), an enzyme that can reduce hydrogen peroxide, organic hydroperoxides, and lipid peroxide, requiring GSH. In the absence of GPX4, uncontrolled lipid peroxidation occurs through the accumulation of lipid radicals, lipid peroxy radicals and then Fenton-mediated lipid alkoxyl radicals. DFO, desferrioxamine, binds free iron preventing the Fenton reaction; Vit E, vitamin E, is a lipophilic anti-oxidants; NFGA, nordihydroguaiaretic acid, is lipoxygenase inhibitor

ferroptosis;[51] (2) α-tocopherol a lipid soluble anti oxidant blocks ferroptosis;[53] (3) enzymes that detoxify 4-HNE are upregulated in cells that are less sensitive to ferroptosis inducers;[60] (4) Ferrostatin-1, a small molecule inhibitor of ferroptosis, which inhibits the oxidation of polyunsaturated fatty acids (PUFAs) by scavenging lipid-free radicals;[61] (5) deuterated PUFAs, which are oxidized far less efficiently than PUFAs, protect cells from ferroptosis showing that PUFA peroxidation is a key event in ferroptosis.[62] Most recently, oxidation of phosphatidylethanolamines was shown during ferroptosis.[63] Reducing the levels of phosphatidylethanolamines by blocking ACSL4, an enzyme involved in phosphatidylethanolamine production, prevented ferroptosis.[64]

Cell death induced by an excess of the neurotransmitter Glu, resulting in inhibition of the system xc$^-$ Cys/Glu antiporter and GSH depletion, was classified as oxytosis or excitotoxicity in neuronal cells.[18] Inhibition of the antiporter reduces the level of intracellular L-Cys required for GSH synthesis. The drop in GSH levels results in activation 12-lipoxygenase (LOX12) and LOX15. LOX12/15 can increase mitochondrial ROS production[65] and an increase in cyclic GMP. Cyclic GMP opens cGMP-gated channels on the plasma membrane, allowing calcium influx.[18] Despite the mechanistic similarities between oxytosis and ferroptosis,[63] which include the dependence on inhibition of the system xc$^-$ Cys/Glu antiporter, a decrease in GSH levels and the presence of lipid peroxidation, the role of calcium in ferroptosis is still matter of debate and α-tocopherol does not block oxytosis.[13,66,67] More experimental evidence is required to substantiate this distinction.

Recently, it was demonstrated that cell death induced by heat (55°C) in *Arabidopsis thaliana* is dependent on iron and involves glutathione drop and lipid ROS, three key ferroptosis-like features. This is probably due to a decrease in the reduction capacity of the cell (glutathione and ascorbate

Table 1 Genes that are positive regulators of ferroptosis

Gene	shRNA knockdown reduces ferroptosis	
	Description	**Function (GeneCards)**
IREB2	Iron-responsive element binding protein 2[51]	RNA-binding protein that binds to iron-responsive elements (IRES), which are stem-loop structures found in the 5′-UTR of ferritin, and δ-aminolevulinic acid synthase mRNAs, and in the 3′-UTR of transferrin receptor mRNA.
ACSF2	Acyl-CoA synthetase factor 2[51]	Mitochondrial LC-fatty-acid beta oxidation.
ATP5G3	ATP synthase F(0) complex subunit C3[51]	Mitochondrial ATP synthase synthesis.
VDAC3	Voltage-dependent anion channel[51]	Integral outer mitochondrial membrane protein that conduct ATP and other small metabolites.
CS	Citrate synthase[51]	Catalyses the synthesis of citrate from oxaloacetate and acetyl coenzyme A in Krebs cycle.
RPL8	60 S ribosomal protein L8[51]	Involved in protein synthesis.
SLC38A1	Solute carrier family 38 member 1[51]	Transporter of glutamine an intermediate in the detoxification of ammonia and the production of urea.
SLC1A5	Solute carrier family 1 member 1[51]	Sodium-dependent neutral amino acids transporter that has a broad substrate specificity, including glutamine, asparagine, and branched-chain and aromatic amino acids.
GLS2	Glutaminase-2[51]	Mitochondrial phosphate-activated glutaminase catalyses the hydrolysis of glutamine to stoichiometric amounts of glutamate and ammonia.
GOT1	Glutamic-oxaloacetic transaminase 1[51]	GOT has a role in amino-acid metabolism, the urea, and Krebs cycles.
ACSL4	Acyl-CoA synthetase long-chain family member 4[84,85]	Converts free long-chain fatty acids into fatty acyl-CoA esters, and thereby have a key role in lipid biosynthesis and fatty-acid degradation. This isozyme preferentially utilizes arachidonic acid as substrate.
LPCAT3	Lysophosphatidylcholine Acyltransferase 3[84]	Insertion of acylated arachidonic acid into membranes.
CARS	cysteinyl-tRNA synthetase[86]	Class 1 aminoacyl-tRNA synthetase, cysteinyl-tRNA synthetase. It catalyzes the aminoacylation of a specific tRNA or tRNA iso accepting family with the cognate amino acid.
LOX12/15	Lipooxygenase 12/15[71]	Non-heme iron-containing dioxygenase that catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators.
AIF	NADH oxidase[71]	Functions both as NADH oxidoreductase and as regulator of apoptosis.
SAT1	Spermidine/Spermine N1-acetyltransferase 1[80]	Catalyses the acetylation of spermidine and spermine, and is involved in the regulation of the intracellular concentration of polyamines and their transport out of cells.
PHGDH	Phosphoglycerate dehydrogenase[51]	NADPH-generating pentose phosphate pathway.
PGD	Glucose-6-phosphate dehydrogenase[51]	NADPH-generating pentose phosphate pathway.

levels) following heat-stress affecting the biosynthesis systems.[68] The occurrence of ferroptosis in plants illustrates that ferroptosis is a common cell death modality in life between animals and plants, but also that a physico-chemical insult such as heat affecting the reduction capacity of the cell can result in ferroptosis.

The near omni-presence of iron and oxygen in the environment and the consequences of failing to control oxidation[69] has determined that GSH and mechanisms to detoxify reactive oxygen species are present in the three domains of life (Archaea, Bacteria, Eukarya). As ferroptosis occurs when control of these protective mechanisms is lost, it can be expected to be a common cell death modality shared in all types of life ranging from bacteria, fungi, plants and animals. This is in marked contrast to the other regulated cell death modalities.

Ferroptosis regulation

Dixon *et al.*[51] and others (summarised in Tables 1 and 2) identified specific genes whose altered expression correlated with increased or decreased sensitivity to ferroptosis. The identity of these genes suggests that one significant way to gain protection from ferroptosis is to regulate genes that control intracellular iron levels[51,70] or to upregulate genes that provide cysteine, so maintaining glutathione levels for longer.[51] This is presumably an adaptive response to inhibition of System xc^-, a glutamate/cysteine antiporter. Another adaptive mechanism is to upregulate genes involved in detoxifying the products of lipid peroxidation.[60]

Lipid peroxidation can occur both non-enzymatically and enzymatically, catalysed by lipooxygenases (LOX). RNAi against LOX and pharmacological inhibition of their activity can protect against erastin-induced ferroptosis and ferroptosis caused by loss of GPX4.[71] Inhibition of ferroptosis by pharmacological inhibitors of kinases, such as U0126 (MEK),[51] and inhibitors of PI3K*α* and Flt3[72] have also been used to argue that ferroptosis is a regulated cell death. RNAi against Flt3 protect against ferroptosis and prevents lipid peroxidation supporting a role for this kinase in ferroptosis. FLT3 can increase ROS by activation of p22phox,[73] which might suggest an indirect effect by decreasing the levels of oxidative stress. RNAi against PI3K*α* does not mimic the effect of the PI3K*α* inhibitor, suggesting that the pharmacological effect is unrelated to PI3K*α* inhibition.[72] At last, U0126 blocks H_2O_2-induced cell death via an anti-oxidant mechanism unrelated to its inhibition of kinase activity casting doubt on any putative role for MEK in ferroptosis.[74]

It appears that changes in genes that affect ferroptosis alter the level of lipid peroxidation rather than the cell's response to lipid peroxidation. Thus, ferroptosis occurs because of

Table 2 Genes that are negative regulators of ferroptosis

Increased expression associated with decreased ferroptosis		
Gene	**Description**	**Function (GeneCards)**
PHGDH	Phosphoglycerate dehydrogenase[86]	Increased expression is associated with increased transsulfuration.[a]
PSAT1	Phosphoserine aminotransferase 1[86]	Associated with increased transsulfuration.[a]
PSPH	Phosphoserine phosphatase[86]	Associated with increased transsulfuration.[a]
CBS	Cystathionine-beta-synthase[86]	Associated with increased transsulfuration.[a]
AKR1C1	Aldo-keto reductase family 1 member C1[60]	Detoxification of 4-hydroxynonenal.[b]
AKR1C2	Aldo-keto reductase family 1 member C2[60]	Detoxification of 4-hydroxynonenal.[b]
AKR1C3	Aldo-keto reductase family 1 member C3[60]	Detoxification of 4-hydroxynonenal.[b]

[a]siRNA against CARS induces enzymes of the transsulfuration pathway, providing an alternative source of cysteine. The transsulfuration pathway is a metabolic pathway involving the interconversion of cysteine and homocysteine, through the intermediate cystathionine. Transsulfuration is an important source of sulfur for glutathione production.
[b]These enzymes catalyse the conversion of aldehydes and ketones to their corresponding alcohols by utilizing NADH and/or NADPH as cofactors.

Table 3 Examples of toxicants with a ferroptotic profile

Toxicant	Toxicity associated with lipid peroxidation	Toxicity inhibited by	
		Iron chelation	***α*-tocopherol**
6-hydroxydopamine	Monteiro & Winterbourn, 1989[87]	Ben-Shachar *et al.*, 1991[88]	Cadet *et al.,* 1989[89]
Anthracyclines	Sawyer *et al.*, 2010[90]	Herman *et al.*, 1994;[91] Henriksson & Grankvist, 1988[92]	Stuart *et al.*, 1978[93]
Paraquat	Bus *et al.*, 1976[94]	Van der Wal *et al.*, 1992[95]	Suntres & Shek, 1995[96]
Methyl mercury	Yonaha *et a*l., 1983;[97] Lin *et al.*, 1996[98]	LeBel *et al.*, 1992[99]	Andersen & Andersen, 1993[100]
Bromobenzene	Fraga *et al.* 1989[101]	Casini *et al.,* 1987[102] Coleman *et al.,* 1990[103]	Maellaro *et al.*, 1990[104]
Carbon tetrachloride	Hashimoto *et al.*, 1968[105]	Younes & Siegers, 1985[106]	Miyazawa *et al.*, 1990[107]
Cadmium	Stacey *et al.,* 1980[108]	Yiin *et al.*, 2001[109]	Fariss 1991[110]
Cephalosporins	Cojocel *et al.,* 1985[111]	Cojocel *et al.,* 1985[112]	Cojocel *et al.,* 1985[112]

dysregulated cellular reduction capacity that protect cells from oxidative stress that could damage cellular components rather than activation of the sort of cell death machinery associated with apoptosis, necroptosis or pyroptosis. A diverse range of signals from heat shock to chemical insult to receptor activation by their cognate ligands can be transduced into the formation of large protein complexes which in turn initiate cell death processes as is the case for the DISC or the apoptosome in case of apoptosis, the necrosome in the case of necroptosis and the inflammasome in the case of pyroptosis. So far, there is no evidence that cellular signalling regulates the formation of protein complexes that are required for the initiation of ferroptosis.

Does ferroptosis fulfil a biological function?

There is good evidence that ferroptosis plays roles in pathological situations. For example, ferroptosis is seen in ischaemia–reperfusion injury,[16] in mouse models of hemochromatosis[75] and it might contribute to cell death during glutamate-induced excitotoxicity. Well-known human toxicants cause lipid peroxidation and their toxicity is reduced by iron chelation and *α*-tocophorol (see examples in Table 3). This profile matches that of ferroptosis inducers, suggesting that these toxicants act by ferroptosis and that preventing ferroptosis may limit the toxicity of these agents. A clearer understanding of ferroptosis may provide new approaches to reduce the harm caused by such toxicants. However, it is not clear that these are situations in which the anti-oxidant defenses are sabotaged as part of a biological program.

Evidence for a biological function may come from studies of the TP53 tumour suppressor.[76] A number of different TP53-regulated processes have been proposed to explain its tumour suppressor role including induction of cell cycle arrest, cellular senescence, apoptosis, and changing cancer cell metabolism. TP53 3KR, a mutant that does not induce cycle arrest, cellular senescence, or apoptosis but still suppresses tumour formation (Figure 3).[77] Jiang *et al.* showed that TP53 3KR inhibits cystine uptake and sensitizes cells to ferroptosis by repressing expression of SLC7A11.[76] The mechanism of action appears to be direct as TP53 binds to the promoter of SLC7A11 in both mouse and human cells. Loss of TP53 acetylation at K98 prevents TP53 regulation of SLC7A11 and also abolishes tumour ferroptosis and suppression.[78] SLC7A11 overexpression reduces ferroptosis and it is also highly expressed in many human tumours, which is perhaps an adaptive response to the increased sensitivity of tumours to systemic depletion or cysteine or cysteine.[79] Further evidence comes from the observation that TP53 also activates SAT1, a gene involved in polyamine synthesis and whose expression can induce lipid peroxidation and induce ferroptosis,[80] although SAT1 has not yet been shown to be a tumour suppresser. This is at this

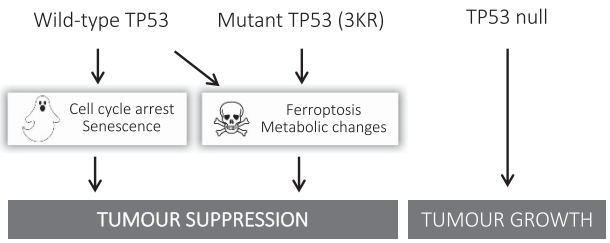

Figure 3 Oncogene activation drives inappropriate cell proliferation that is countered by the tumour suppressor activity of TP53, which involves several distinct mechanisms. In the absence of TP53, tumour growth is unchecked. The 3KR TP53 mutant activates only a subset of TP53 responses but is still able to suppress tumour growth, showing that TP53-induced metabolic changes involved in energy production[77] and/or TP53-induced ferroptosis are sufficient to inhibit tumour growth[76]

moment the only evidence of a signal transduction pathway linking a molecular change (activation of a tumour suppressor) to execution of the ferroptotic process.[7]

It is worth remarking on the differences between mice and men in the context of these experiments. TP53 null mice develop thymomas and sarcomas, but not carcinomas, which are the majority of tumours afflicting humans. Transgenic mouse models with different TP53 mutations (e.g., TP53 R270H) better reflect human disease[81] but whether TP53-induced ferroptosis is important for suppressing these tumours has not been tested. However, a link between an African-specific TP53 polymorphism, tumour suppression and ferroptosis was recently reported.[82] A polymorphism at TP53 codon 47 (S47) has only slight effects on apoptosis but dramatically reduces ferroptosis. Although how the polymorphism affects the incidence or type of human cancer is not known, both homozygous and heterozygous transgenic mice show increased tumour formation including hepatocellular carcinoma.

Together these data suggest that ferroptosis causes tumour suppression. This would be good evidence that ferroptosis is indeed a regulated cell death with an important functional context, but at this stage an *in vivo* tumour suppressor role for SLC7A11 repression has not been demonstrated and a role for the other TP53-mediated metabolic changes have not been excluded. Experiments testing whether TP53 down-regulation of SLC7A11 is required for tumour suppression would help resolve this question. Such experiments are complicated by the different and potentially redundant tumour suppressor mechanisms activated by TP53 and should probably be attempted in the TP53 3KR mutant background where some of the other TP53-regulated pathways are inactivated.

The recent finding that plants display ferroptosis induction following heat-stress[68] and produce compounds that block ferroptosis[83] opens an intriguing possibility that ferroptosis may represent an adaptive response to stress and infection. As is the case for heat-stress, pathologic ischemia conditions may lead to depletion of GSH levels resulting in enhanced sensitivity to iron- and lipid peroxidation induced cell death, which could be part of a defensive response against infection.

Conclusion

In this brief description of cell death modalities, we draw parallels between the apoptosis, pyroptosis, necroptosis, and ferroptosis, and conclude that ferroptosis does not share characteristics found in the other three cell death modalities. Ferroptosis is not yet known to involve the formation of death-inducing protein complexes (DISC, apoptosome, necrosome, inflammasome) or the activation of effector proteins (caspase-3, MLKL, gasdermin) that kill the cell in response to specific extracellular signals or intracellular stimuli following organelle damage. Instead it is cell death that occurs when anti-oxidant defences are overcome and key cellular macromolecules are chemically damaged. The evidence that ferroptosis has a biological purpose is still limited, whereas apoptosis, pyroptosis, and necroptosis serve clear physiological functions during development, homeostasis, inflammation and infection. As ferroptosis apparently occurs during ischemia–reperfusion conditions its biological function may be sought in that context.[16] New evidence corroborating the proposed role of ferroptosis in tumour suppression would greatly strengthen the conclusion that this form of cell death is also regulated and functions in an evolutionary selectable context. Ferroptosis could also be of functional importance in conditions of oxidative stress, decreased reduction capacity in cells or upon excessive levels of free iron. A better understanding of the occurrence of these conditions and the possible connection with ferroptosis will undoubtedly boost potential treatments of a broad range of diseases.

Conflict of Interest

The authors declare no conflict of interest.

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