

Research Highlight

Ferroptosis: a novel cell death form will be a promising therapy target for diseases

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Recently, Friedmann Angeli *et al.* [1] reported that the loss of ferroptosis regulation enzyme glutathione peroxidase 4 (GPX4) will cause an overwhelming ferroptosis of renal cells, which eventually induces renal failure. Yet, liproxstatin-1, a novel potent ferroptosis inhibitor, is able to alleviate tissue injury of ischemia/reperfusion-induced renal injury. This study smartly expanded the research on nonapoptotic cell death, ferroptosis, as researchers were just focused on its effect on tumor and neuronal diseases before [2,3]. Actually, ferroptosis is related to multiple pathophysiological processes, and triggering or inhibiting ferroptosis will be novel therapy strategies for many diseases, such as atherosclerosis, angiocardopathy, and diabetes. In their research, the complex lipid oxidation was also investigated, which provided new possibilities for redox-target therapy [1].

Apoptosis has been considered as the major form of cell death, but sometimes target apoptosis cannot achieve satisfactory therapeutic effect on tumor and other diseases [4]. Consequently, nonapoptotic cell death processes have continuously been explored and discussed, including necroptosis and ferroptosis. In 2012, Dixon *et al.* [5] found for the first time that the oncogenic RAS-selective lethal compounds can trigger a unique iron-dependent form of nonapoptotic cell death, ferroptosis which is a totally new pattern of cell death. It is different from apoptosis, autophagy, and necrosis in morphology (smaller mitochondrial with increased membrane density), biochemistry, and genetics. Furthermore, it is specifically associated with iron and characterized by distinctive lipid oxidation, and expands the study on the network of nonapoptotic cell death.

With further research on ferroptosis, the occurrence and regulation mechanism of ferroptosis has gradually been revealed (Fig. 1) [1, 5–9]. Factors involved in the iron metabolism regulation system, such as transferrin (TF)-receptor (TFR) and divalent metal transporter 1, are activated to induce iron accumulation, and then fenton reaction is evoked during ferroptosis. Heat shock protein beta-1 (HSPB1) was found to be a negative ferroptosis regulator which can reduce iron accumulation in cancer cells [6]. Although Dixon *et al.* [5] have demonstrated that NADPH oxidases provide one source of reactive oxygen species (ROS) during ferroptosis, the general ROS production pathway is still unknown, because it is independent of the major ROS generator, the mitochondrial electron transport chain. The cystine/glutamate transporter system x_c^- plays an important role in

maintaining cellular glutathione (GSH) and redox equilibrium, and some ferroptosis inducers were found to promote ROS production by depressing system x_c^- or restricting the anti-oxidant effect of GPX4, an enzyme that is inactivated by GSH depletion [7]. In addition, p53 inhibits cystine uptake and promotes ROS-induced stress by repressing the key system x_c^- component, SLC7A11, suggesting a novel anti-cancer mechanism of this tumor suppressor gene [8]. Sorafenib, a multikinase inhibitor and effective anti-tumor drug, was found to have cytotoxic effects on the hepatocellular carcinoma (HCC) cells through triggering the iron-dependent oxidative cell death, ferroptosis. And this form of cell death is enhanced in retinoblastoma (Rb) protein-negative HCC cells [9]. Thus, blocking the activities of antioxidant, system x_c^- , GSH synthetase, and GPX4, or increasing the concentration of iron would induce extremely aberrant ROS production and lipid peroxidation, the high-risk factors for inducing ferroptosis [1]. Accordingly, a series of ferroptosis inducers and inhibitors have been developed and tested (Table 1) [1,5,10–12]. These are useful tools for further study of the specific mechanism and process of ferroptosis.

Considering that the change of mitochondria was the major morphology difference in those multiple types of cell death and that GPX4, an essential ferroptosis regulator gene with the six mitochondria genes exhibiting abnormal expression during ferroptosis [5], locates in mitochondrion [7], we believe that mitochondria may play a central role in ferroptosis. Some researchers have showed that mitochondrial oxidation is not the lethal factor for ferroptosis, and the outer mitochondrial membrane rupture just represents the irreversible ferroptosis [1,5]. How does the contradiction emerge? The crucial target for oxidation is still unknown at present. Besides mitochondria, other sites of ROS production and accumulation may also be important for ferroptosis. During ferroptosis, the endoplasmic reticulum (ER) and attached ribosome may be the first damaged sites which then transmit the damage signal to the mitochondria. Just as in photodynamic stress therapy, the initial ROS stimulate reticulophagy by impairing ER, and this oxidative damage is rapidly conveyed to the mitochondria and causes cell death [13]. ROS, as important signaling molecules, are involved in multiple pathologic processes, such as autophagy. Whether the compensatory action autophagy can be arisen to remove ferroptosis injured cells? How does this cross-talk occur? These are important questions to be answered in the future.

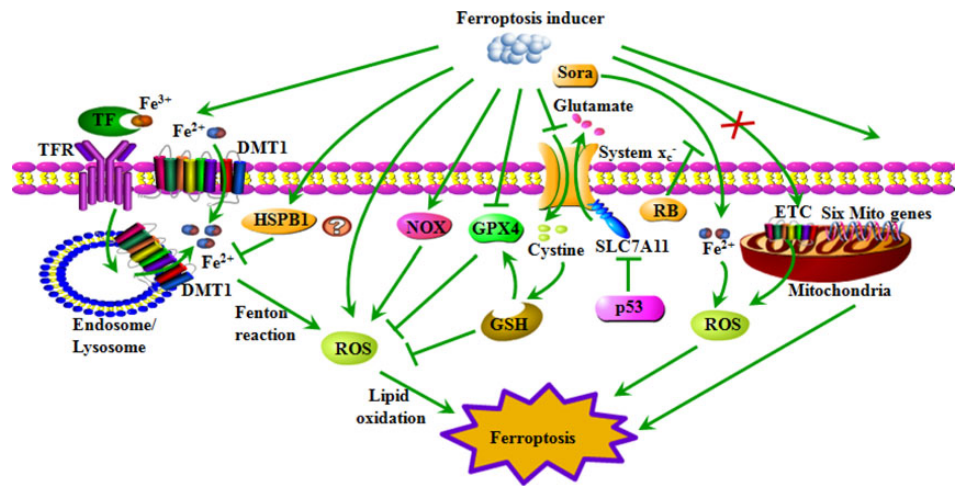


Figure 1. The occurrence and regulation mechanism of ferroptosis Cystine: ; divalent metal transporter 1 (DMT1): ; endosome/lysosome: ; electron transport chain (ETC): ; Fe²⁺: ; Fe³⁺: ; glutamate: ; glutathione peroxidase 4 (GPX4): ; glutathione (GSH): ; heat shock factor binding protein 1 (HSPB1): ; NADPH oxidase (NOX): ; reactive oxygen specials (ROS): ; mitochondria: ; p53: ; retinoblastoma (RB) protein: ; sorafenib (Sora): ; SLC7A11: ; system x_c⁻: ; transferrin: ; transferrin receptor (TFR): ; six mito genes (RPL8, IREB2, ATP5G3, CS, TTC35, and ACSF2): ; unknown ROS production pathway: .

Table 1. List of inducers, inhibitors and regulators of ferroptosis

	Class	Name	Characteristic
Inducer	Class I	Erastin, Erastin derivatives (MEII, PE, AE), DPI2, BSO, SAS, β-ME, glutamate, lanperisone	Indirect inhibit GPX4 (via depletion GSH)
	Class II	RSL3, DPI7, DPI10, DPI12, DPI13, DPI17, DPI18, DPI19, ML162	Inhibit GPX4 directly
Inhibitor	Drug	Sorafenib	Induce oxidative stress, reduce Rb
	Fer-1 and related analogs	ferrostatin-1, SRS8-24, SRS8-72, SRS11-92, SRS12-45, SRS13-35, SRS13-37, CA-1	Do not inhibit the MEK/ERK pathway, but ROS accumulation
	Spiroquinoxalinamine derivative	Liproxstatin-1	Unknown mechanism
	Anti-oxidant	Trolox, U0126, vitamin E	Inhibit ROS accumulation and ROS oxidative
	Iron inhibitor	DFO, CPX, 2,2-bipyridyl	Antagonize iron
Regulator	Others	CHX, AOA, Ebs	Inhibit protein synthesis or transaminase, promote GPX
		RPL8, IREB2, ATP5G3, CS, TTC35, ACSF2	Mitochondrial genes
		GPX4	Essential regulator
		P53, HSPB1, Rb, SLC7A11	Cancer regulator

Iron overload can induce ROS production, oxidation of the lipid, protein and DNA, and trigger or mediate cell death. However, the cell death patterns have not been particularly explored. The discovery of ferroptosis partially elucidates the iron-related cell death. Ferroptosis can be prevented by treatment with iron chelator or interference with iron metabolism regulator, such as iron response element binding protein 2 (IREB2) [5]. Thus, iron metabolism-related proteins are crucial for the study of ferroptosis. Previous studies have indicated that both iron deficiency and iron overload are harmful to human health. High ferrous in the heart is easy to cause myocardial dysfunction and metabolism injury, and eventually lead to heart disease [14,15]. Iron overload is closely related to many metabolism diseases because it can stimulate lipid oxidation and tissue damage, leading to atherosclerosis and diabetes. Abnormal iron accumulation is often found in tumor and neuronal diseases, which are prone to induce ferroptosis. So, further studies are needed to clarify whether ferroptosis plays a role in

these iron deposition-related diseases and whether it can serve as a therapeutic target?

In brief, the discovery of the ferroptosis in normal cells is a great progress in the research of cell death. Lipid hydroperoxides are described as the driving force and iron as the catalyst for ferroptosis. Thus, identifying the ROS target sites and understanding the detailed mechanism of lipid oxidation and iron action will be beneficial to the treatment of iron overload-related diseases, such as atherosclerosis, cardiovascular diseases, and diabetes by targeted ferroptosis interference.

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References

1. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, *et al.* Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol* 2014, 16: 1180–1191.
2. Galmiche A, Chauffert B, Barbare JC. New biological perspectives for the improvement of the efficacy of sorafenib in hepatocellular carcinoma. *Cancer Lett* 2014, 346: 159–162.
3. Speer RE, Karuppagounder SS, Basso M, Sleiman SF, Kumar A, Brand D, Smirnova N, *et al.* Hypoxia-inducible factor prolyl hydroxylases as targets for neuroprotection by ‘antioxidant’ metal chelators: from ferroptosis to stroke. *Free Radic Biol Med* 2013, 62: 26–36.
4. Fulda S, Vucic D. Targeting IAP proteins for therapeutic intervention in cancer. *Nat Rev Drug Discov* 2012, 11: 109–124.
5. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, *et al.* Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012, 149: 1060–1072.
6. Sun X, Ou Z, Xie M, Kang R, Fan Y, Niu X, Wang H, *et al.* HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene* 2015, [Epub ahead of print]
7. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, *et al.* Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014, 156: 317–331.
8. Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, Baer R, *et al.* Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 2015, 520: 57–62.
9. Louandre C, Marcq I, Bouhlal H, Lachaier E, Godin C, Saidak Z, François C, *et al.* The retinoblastoma (Rb) protein regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma cells. *Cancer Lett* 2015, 356: 971–977.
10. Skouta R, Dixon SJ, Wang J, Dunn DE, Orman M, Shimada K, Rosenberg PA, *et al.* Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *J Am Chem Soc* 2014, 136: 4551–4556.
11. Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, *et al.* Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *Elife* 2014, 3: e02523.
12. Shaw AT, Winslow MM, Magendantz M, Ouyang C, Dowdle J, Subramanian A, Lewis TA, *et al.* Selective killing of K-ras mutant cancer cells by small molecule inducers of oxidative stress. *Proc Natl Acad Sci USA* 2011, 108: 8773–8778.
13. Rubio N, Coupienne I, Di Valentin E, Heirman I, Grooten J, Piette J, Agostinis P. Spatiotemporal autophagic degradation of oxidatively damaged organelles after photodynamic stress is amplified by mitochondrial reactive oxygen species. *Autophagy* 2012, 8: 1312–1324.
14. Gammella E, Recalcati S, Rybinska I, Buratti P, Cairo G. Iron-induced damage in cardiomyopathy: oxidative-dependent and independent mechanisms. *Oxid Med Cell Longev* 2015, 2015: 230182.
15. Xie F, Liu W, Feng F, Li X, Yang L, Lv D, Qin X, *et al.* A static pressure sensitive receptor APJ promote H9c2 cardiomyocyte hypertrophy via PI3K-autophagy pathway. *Acta Biochim Biophys Sin* 2014, 46: 699–708.