

Glutathione peroxidase 4 (Gpx4) and ferroptosis: what's so special about it?

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The system X_C^- /glutathione/glutathione peroxidase 4 (Gpx4) axis pivotally controls ferroptosis, a recently described form of regulated non-apoptotic cell death. Compelling evidence has established that this route of cell death is not only of high relevance for triggering cancer cell death, but also proves to be amenable for therapeutic intervention to halt ischemia/reperfusion-related diseases.

Glutathione peroxidase 4 (Gpx4) is 1 of 8 known glutathione peroxidases in mammals. With the exception of Gpx5, Gpx7, and Gpx8 (and Gpx6 in rodents), glutathione peroxidases (Gpx) are selenoproteins.¹ Selenoproteins contain the 21st amino acid selenocysteine, which differs from cysteine by a single atom, selenium replacing for sulfur. Although the advantage of using selenocysteine instead of cysteine is not really understood, it is generally believed that selenocysteine provides higher reactivity and superior efficiency in redox reactions. All members of the Gpx family share the same basic function of the reduction of peroxides at the expense of glutathione (GSH), or other thiol containing compounds in the case of the monomeric Gpx4, Gpx7, and Gpx8. Despite structural and catalytic similarities, Gpx4 is unique among Gpx isoforms as it is the only enzyme capable of reducing esterified oxidized fatty acids and cholesterol hydroperoxides. The relevance of this unique function is highlighted by *in vivo* studies using knockout (KO) models for this enzyme. The first KO of *Gpx4* was reported in 2003² and found to cause early embryonic lethality. Therefore, investigations into Gpx4 functions in adult animals are only possible using conditional KO systems.

In 2008, we generated the first animal carrying a conditional *Gpx4* allele.³ Using mouse embryonic fibroblasts with an inducible *Gpx4* disruption, we were able to demonstrate that a non-apoptotic form of cell death is elicited upon *Gpx4* deletion and that this cell death is preceded by lipid oxidation.³ Subsequent studies with these animals revealed that Gpx4 is essential for maintaining tissue homeostasis by preventing cell demise and tissue damage in several organs including brain, skin, and endothelium.^{3,4} Nevertheless, some tissues did not appear to be directly affected by loss of Gpx4, such as the heart (unpublished observation), indicating that specific conditions might be required to trigger this type of cell death.

In 2012, Stockwell and colleagues described a novel form of cell death that was elicited by inhibiting the cystine/glutamate antiporter, system X_C^- , a transporter that feeds cells with cysteine to be used for protein and, in particular, GSH biosynthesis⁵ (Fig. 1). Following up on this, the same group identified the most important GSH-dependent enzyme in this pathway as GPX4.⁶ Yet these studies have largely focused on the therapeutic possibility of triggering this type of cell death in cancer cells, leaving the option of pharmacological inhibition of this

pathway in a relevant pathological situation unaddressed. With this in mind, we have generated animals with inducible deletion of *Gpx4* as an ideal tool to reveal the adult tissues most sensitive to ferroptosis-relevant cell death. We found that the animals died within 2 weeks of Gpx4 loss as a result of massive renal tubule cell death and acute kidney failure.⁷ Pharmacological targeting of this pathway *in vivo* was further shown to be possible by the development of a novel class of drug-like small molecules named liproxstatins that are able to extend the survival of *Gpx4* null mice by approximately 35%. Further evidence that ferroptosis could indeed be targeted *in vivo* under pathologically relevant conditions was independently provided by 2 groups, who showed that liproxstatin and a second generation of ferrostatins mitigated tissue damage in preclinical models of liver and kidney ischemia/reperfusion damage, respectively.^{7,8}

Despite these recent advances in this intriguing field, questions concerning the downstream mechanisms in the response to *Gpx4* deletion have remained largely unanswered. Our recent work now shows that one of the earliest events upon loss of Gpx4 *in vivo* is cardiolipin oxidation, spreading from there to other classes of

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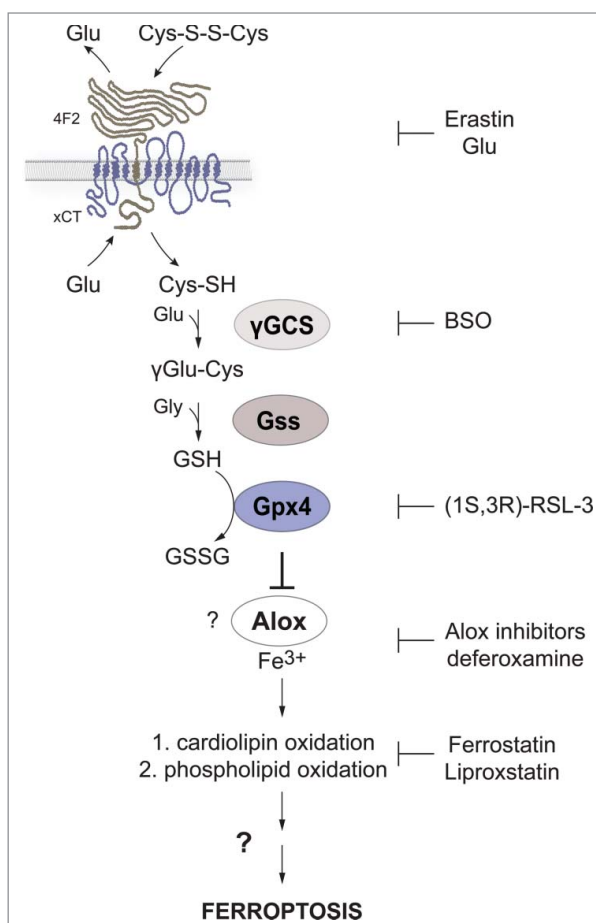


Figure 1. Biochemical events in the upstream control of ferroptosis. The upstream events controlling ferroptotic signaling are depicted. Cystine, the oxidized form of cysteine (Cys-S-S-Cys), is taken up by the cystine/glutamate antiporter that consists of the 4F2 (Slc3a2) heavy chain and xCT (Slc7a11) light chain. Upon intracellular reduction, cysteine, along with glutamate and glycine, are used for protein and glutathione (GSH) biosynthesis. GSH is synthesized in 2 consecutive steps by the heterodimer γ -glutamylcysteine synthetase (γ GCS consists of Gclc and Gclm) and glutathione synthetase (Gss, GS), each step requiring one molecule of ATP. Glutathione peroxidase 4 (Gpx4) is a central GSH-utilizing enzyme and counteracts lipoxygenase (Alox) activities and phospholipid/cardioplipin oxidation events. In the absence of Gpx4, cardioplipin oxidation followed by phospholipid peroxidation causes ferroptosis through still unknown mechanisms. Small-molecule inhibitors of the different players are shown on the right. Abbreviations: BSO, L-buthionine sulfoximine; GSSG, oxidized glutathione; γ Glu-Cys, γ -glutamylcysteine.

phospholipids such as phosphatidylethanolamine and phosphatidylcholine.⁷ This feature seems to be clearly distinct from apoptosis, in which only cardiolipin oxidation via the peroxidase function of cytochrome c (cytochrome c, somatic; Cyto) has been demonstrated.⁹ Although the reasons for this are still unclear, one may speculate that Gpx4 is required to allow a “silent” form of cell death that could

tentatively switch upon Gpx4 loss/malfunction to a highly inflammatory cell death modality culminating in the release of several proinflammatory lipid mediators.⁷ What happens to cells undergoing apoptosis under Gpx4-compromised conditions is still not known and warrants further investigation.

Another point that deserves attention is how a ferroptotic-like cell death can

be induced in an ischemia/reperfusion scenario. It has been reported that proper Gpx4 function is required to inhibit cell death in kidney tubule cells yet this phenotype is observed only under complete Gpx4 loss, which is most likely not the case under a pathologically relevant setting. Notwithstanding, ferroptosis inhibitors present a beneficial effect during ischemia/reperfusion, indicating the engagement of this pathway under these conditions.^{7,8} Therefore, it seems plausible that Gpx4 function and/or expression might be modulated under such conditions, for example by post-translational modifications that would directly influence its catalytic activity such as oxidative degradation by oxygen radicals or proteasome-mediated degradation. Although still speculative, these hypotheses are not without foundation as similar events have already been reported for some other redox-related enzymes. A second consideration is that this modification would most likely occur at a specific site; we tentatively speculate that such events would likely take place at the inner membrane space of mitochondria, as we have shown that eliciting ferroptosis disrupts the outer mitochondrial membrane.⁷ Moreover, this was also identified as the major site of oxygen radical production under ischemic conditions through re-oxidation of succinate via the succinate dehydrogenase complex during reverse electron transport at mitochondrial complex I.¹⁰

We believe that a better understanding of how Gpx4 function is modulated under particular settings such as ischemia/reperfusion and tumorigenesis, and how downstream events orchestrate cellular demise under ferroptotic conditions, will allow us to efficiently prevent and/or trigger this form of cell death in therapeutically responsive scenarios.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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