

# Chapter 43

## Glutathione Peroxidase 4 and Ferroptosis

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**Abstract** Glutathione peroxidase 4 (Gpx4) is one of eight members of the mammalian glutathione peroxidase family of enzymes. Gpx4 is unique due to its capacity to efficiently reduce phospholipid hydroperoxides. Additionally, it has been recognized that Gpx4 governs a novel form of non-apoptotic cell death, named ferroptosis. Ferroptosis was initially described to be induced by small molecules in specific tumor types and in engineered cells overexpressing oncogenic RAS. Recently, its relevance in non-transformed cells and tissues was highlighted expanding the importance of this cell death pathway in several pathophysiological contexts, encompassing immunity, neurodegenerative diseases and tissue damage upon ischemia/reperfusion scenarios. Importantly, regulation of selenoprotein biosynthesis emerges as an important factor in control of ferroptosis. In this chapter, we present an updated view on the ferroptotic process as well as review the implications of how selenoprotein expression might impact on ferroptosis *per se*.

**Keywords** Ferroptotic signaling • Ischemia/reperfusion injury • Lipid peroxidation • Liproxstatin-1 • Mevalonate pathway • Neurodegeneration • Non-apoptotic cell death • PHGPx • Phospholipid hydroperoxide glutathione peroxidase • Regulated necrosis

### 43.1 Introduction

Glutathione peroxidase 4 (Gpx4) is one of eight members of the glutathione peroxidase family of enzymes. Specifically, mammalian Gpx1, Gpx2, Gpx3 and Gpx4 are selenoproteins, whereas Gpx5, 7 and 8 are Cys-containing paralogs. Additionally, GPX6 is a selenoprotein in humans, but a Cys-containing enzyme in rodents and some other mammals [1]. Glutathione peroxidases (Gpxs) are most commonly known for a shared enzymatic mechanism involving a conserved catalytic tetrad, consisting of selenocysteine (Sec), asparagine (Asn), glutamine

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(Gln) and tryptophan (Trp) [2]. Their catalytic activity is responsible for reducing  $H_2O_2$  and organic hydroperoxides to  $H_2O$  and the corresponding alcohols, respectively. The catalytic consumption of peroxides is driven by glutathione (GSH) which serves as electron donor and is then recycled by GSH reductase at the expense of NADPH. Nevertheless, it is now recognized that GSH is not the sole physiological reducing substrate for Gpxs. Some alternative substrates have been shown to be thioredoxin and/or glutaredoxins for Gpx3, protein disulfide isomerase for Gpx7 and 8 [3], and, under special conditions, Gpx4 can use “unspecific” thiols acting as a non-selective thiol peroxidase [4].

Gpx4 was initially characterized in 1982 by Ursini and colleagues and shown to be an enzyme efficiently protecting liposomes and biomembranes from peroxidative degradation in the presence of GSH [5]. Gpx4 presents substantial overall homology to the monomeric Gpx7 and 8, which, however, differ from Gpx4 by the presence of Cys instead of Sec. Compared to other Gpxs, the group of monomeric Gpxs is the least homologous to the most studied “classical” or “cytosolic” Gpx1. Gpx4 differs from other Gpx family members in terms of its monomeric structure, relatively low substrate specificity, and its necessity for mouse development [6].

Analysis of the *Gpx4* gene identified three distinct isoforms which differ by the use of three individual promoters [7]. Each isoform presents a different subcellular localization and a particular tissue-specific expression. Alternative transcription initiation at exon 1 and exon 1b (Ea) generates either the short cytosolic form (20 kDa) or the mitochondrial form (23 kDa) with an N-terminal mitochondrial leader sequence. The alternative exon (Ea) gives rise to a 34 kDa isoform of Gpx4 containing a nuclear targeting sequence. The nuclear form of Gpx4 (nGpx4) was previously referred to as sperm nuclei-specific Gpx (snGpx) [8]. The cytosolic form of Gpx4 is ubiquitously expressed, whereas the mitochondrial and the nuclear forms are predominantly expressed in testis [8–10].

Based on genetic studies in mice, it is now well accepted that the isoform of Gpx4 conferring the cytoprotective function and the one essential for mammalian development is the short (also referred to as cytosolic) form [11]. Specific ablation of the short form is not possible as it is part of both the nuclear and mitochondrial forms. Alternatively, specific ablation of the mitochondrial and the nuclear isoforms is feasible due to their exclusive transcription initiation sequences and consequently the presence of unique N-terminal extensions, i.e., the mitochondrial targeting sequence and the nuclear localization sequence/DNA binding motifs, respectively. Deletion of either the nuclear or the mitochondrial form gives rise to viable offspring, however, the respective knockout (KO) mice exhibit defects in male gametogenesis including male infertility [12, 13]. Additional studies crossing transgenic mice overexpressing the cytosolic or the mitochondrial form on a *Gpx4* KO background demonstrated that only the cytosolic form is able to rescue the lethal phenotype of the mouse *Gpx4* null mutation [11].

Several studies have reported a pro-survival function of Gpx4 in protecting against apoptosis (reviewed in [14]). More recently, Gpx4 overexpression has been shown to prevent tumor necrosis factor  $\alpha$ -induced cell death in quiescent Jurkat T lymphocytes in a mechanism involving decreased formation of truncated

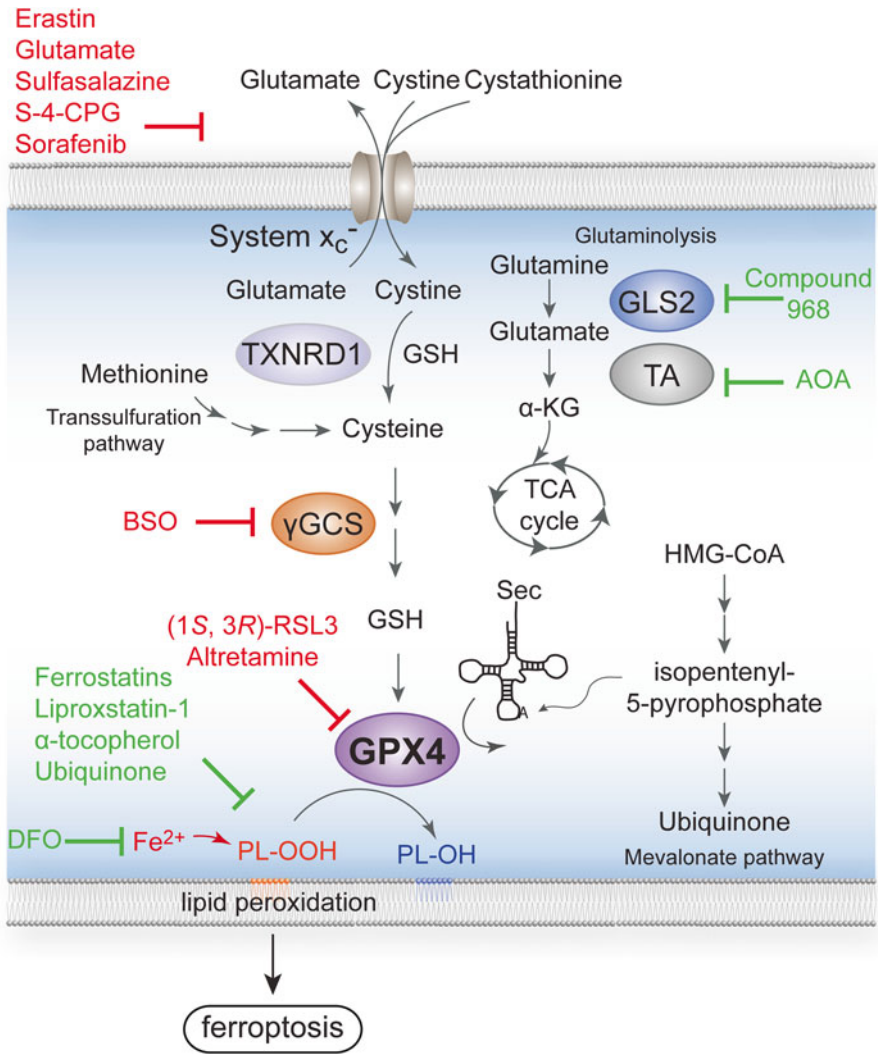
phospholipids [15]. These in turn were generated by the oxidative degradation of phosphatidylcholine through NADPH oxidase 4-derived  $H_2O_2$ . It was also found that Gpx4 has an essential function in protecting against cardiolipin oxidation, thus negatively regulating the intrinsic apoptotic pathway [16]. This notion has been further reinforced by *in vivo* studies showing that overexpression of Gpx4 protects liver from diquat-induced damage by decreasing cardiolipin oxidation and subsequently inhibiting the release of pro-apoptotic factors such as cytochrome C and SMAC/DIABLO. Interestingly, using conditional gene disruption and robust oxilipidomics analysis, it was shown that upon Gpx4 deletion in the kidney of induced Gpx4 KO mice several oxidized products of cardiolipin accumulate and that this process precedes the oxidation of any other class of phospholipids [17].

## 43.2 Gpx4: Beyond Apoptosis

It is now increasingly clear that cells can engage in a plethora of cell death pathways that are not limited to apoptosis. Among the cell death paradigms that have attracted growing interest from the scientific community are necroptosis, ferroptosis/oxytosis, parthanatos and cyclophilin-D mediated necrosis (for a comprehensive review see [18, 19]). Among these novel cell death paradigms, ferroptosis has gained momentum because of its putative implication in several pathological conditions, spanning from ischemia/reperfusion injuries in various tissues to neurodegenerative diseases.

The term “ferroptosis” was first coined in 2012 by Stockwell and co-workers [20] (Fig. 43.1), where they identified the mechanism of action of the small molecule erastin, which had been discovered to be synthetically lethal to isogenic cancer cell lines carrying mutant forms of RAS [21]. Erastin was shown to deprive cells from GSH via abrogation of cystine uptake mediated through inhibition of the system  $x_C^-$  antiporter [20]. This antiporter takes up one molecule of cystine (the oxidized form of Cys) at the expense of the release of one molecule of glutamate [22]. Interestingly, inhibition of system  $x_C^-$  can also be achieved by high extracellular concentrations of glutamate (>1 mM). These observations are reminiscent of previous work demonstrating that glutamate can be neurotoxic by a mechanism that also involves GSH depletion and subsequent calcium-dependent activation of lipoxygenases, culminating in a form of cell death, named oxytosis [23]. At present, oxytosis and ferroptosis are hardly distinguishable, and more studies are warranted to determine if they in fact represent the same phenomenon.

Of note, a recent study has provided provocative evidence that ferroptosis may be involved in antitumor actions of the tumor suppressor p53 through its ability to inhibit transcription of *SLC7A11*, which is the substrate-specific subunit of system  $x_C^-$  [24]. Nonetheless, ferroptosis and oxytosis share similar features: Cys deprivation (by decreased cystine uptake) and GSH depletion. The crucial function of GSH is further supported by studies showing that GSH depletion using the  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) inhibitor, L-buthionine sulfoximine (BSO),



**Fig. 43.1** Gpx4 is the key regulator of ferroptotic cell death. Availability of cysteine either through the cystine/glutamate antiporter (System  $x_c^-$ ), the neutral amino acid transporter (ASCT, not shown) or the transsulfuration pathway feeds glutathione (GSH) biosynthesis. Gpx4 is unique and a central node in ferroptosis as it directly reduces lipid hydroperoxides (PL-OOH) to the corresponding alcohols (PL-OH). Expression of Gpx4 is indirectly dependent on the mevalonate pathway (see text). Besides iron-dependent lipid peroxidation, glutaminolysis was identified as a source of detrimental oxygen radicals. Inducers of ferroptosis are shown in *red*, whereas ferroptosis inhibitors in *green*.  $\alpha$ -KG  $\alpha$ -ketoglutarate, AOA aminooxyacetate, BSO L-buthionine sulfoximine, DFO deferoxamine,  $\gamma$ -GCS  $\gamma$ -glutamylcysteine synthetase, GLS2 glutaminase 2, HMG-CoA 3-hydroxy-3-methylglutaryl coenzyme A, S-4-CPG (S)-4-Carboxyphenylglycine, TA transaminase, TCA tricarboxylic acid, TXNRD1 thioredoxin reductase 1

is able to induce cell death in a manner similar to ferroptosis/oxytosis. The recognition that GSH is essential to prevent this form of cell death came imbued with the intrinsic question: whether there is a GSH dependent enzyme responsible for this pro-survival effect. Definitive answers to this question were given in 2014, when two groups independently provided unequivocal evidence that Gpx4 is the rate limiting enzyme supporting the pro-survival function of GSH, at least in certain defined contexts. Specifically, while investigating new molecules able to elicit ferroptosis, Stockwell and collaborators were able to identify a sub-class of ferroptosis inducers that are able to trigger cell death with ferroptotic characteristics without impacting on GSH levels [25]. Target characterization of their mechanism of action culminated with the discovery of these small molecules as the first Gpx4 inhibitors. Concomitantly, using Gpx4 conditional KO cells, our group was able to demonstrate that cell death induced by Gpx4 deletion can be prevented by ferroptosis inhibitors and that this process proceeds without impacting on GSH levels [17].

Analogous to its function in preventing the accumulation of phospholipid hydroperoxides, the inactivation of Gpx4, either through genetic or pharmacologic means, leads to increased levels of oxidized phospholipids. At present it is, however, not clear if lipid peroxidation is just an executioner of the cell death process by causing loss of physicochemical properties of cellular membranes that will trigger loss of barrier function. Alternatively, lipid hydroperoxides may represent defined and meaningful signals that are transduced by protein sensors engaged downstream, thus activating defined signaling cascades. Additionally, the assumption that Gpx4 inhibition is associated with an elevated level of lipid peroxidation that is required for cell death progression may support the notion that cells constantly oxidize their lipidomes for a yet-to-be defined physiological purpose, a process that, in turn, is finely tuned by Gpx4. Therefore, it is of utmost importance to understand when and how these lipids are being modified. Early work has proposed that lipoxygenases would fulfill the criteria of best candidates to perform such tasks [26, 27], a notion that was further supported by pharmacological means [28]. Nonetheless, recent studies have questioned their importance and provide evidence of off-target effects for most lipoxygenase inhibitors [17].

On the other hand, it has become evident that iron metabolism plays a critical role in ferroptosis [20]. Studies have revealed several molecules involved in iron metabolism that are able to impact on the ferroptotic process. Specifically, iron-bound transferrin (Tfn) taken up via the transferrin receptor (TfnR) is now accepted as an important supplier for the iron pool that can be mobilized during ferroptosis [29]. Moreover, iron responsive element binding protein 2 (IREB2), F-box and leucine-rich repeat protein 5 (FBXL5) have been shown to impinge on this system presumably by stabilizing *Tfn* and *TfnR* mRNAs by IREB2 [30]. The effect of IREB2 is then counteracted by FBXL5 which actively marks IREB2 for proteosomal degradation [31]. Yet, how exactly iron impacts on the cell death process is far from being clear, as it is not yet known whether iron *per se* via mobilization of the so-called labile iron pool promotes unrestrained damage to lipids and other bio-molecules or, alternatively, supports the function of a critical iron-dependent enzyme.

It has recently been proposed that the process of glutaminolysis is essential for ferroptosis and that this can be efficiently prevented by inhibiting the conversion of glutamine to glutamate [29], a process that can be bypassed by supplying  $\alpha$ -ketoglutarate. Interestingly,  $\alpha$ -ketoglutarate is a substrate of  $\alpha$ -ketoglutarate-dependent dioxygenases, a family of iron-dependent enzymes required for the oxygenation of a wide array of substrates which have also been shown to be an important source of mitochondria-derived  $H_2O_2$ . In addition to the contribution of glutamine metabolism to ferroptosis, recent reports have started to demonstrate that lipid metabolism is also a critical node defining sensitivity to ferroptosis. A study using insertional mutagenesis in haploid KBM7 cells was able to identify acetyl-CoA synthase long chain family member 4 and lysophosphatidylcholine acyltransferase 3 as important regulators of cell death induced by the Gpx4 inhibitor RSL3 [32]. Yet, how they modulate ferroptosis is still unclear. At present, it is hypothesized that these proteins modulate the content of arachidonic acid-containing phospholipid species that are modified during ferroptosis. Nonetheless, which and how these phospholipids are oxidatively modified remains unresolved.

The recognition that Gpx4 is a key regulator of ferroptosis raises the question of how Gpx4 activity/expression levels are modulated. Importantly, mechanisms governing transcription/translation of selenoproteins may shed light on some of the processes that could impact on Gpx4. Specifically, early work has shown that the Sec-specific tRNA, designated tRNA<sup>[Ser]<sup>Sec</sup></sup>, may undergo post-translational modifications, which directly affects translational efficiency. Among the critical posttranscriptional modifications of tRNA<sup>[Ser]<sup>Sec</sup></sup> is the Trt1-mediated isopentenyladenosine (i<sup>6</sup>A) [34]. This modification is important for ensuring maximal translation efficiency at the UGA codon. A critical feature of this system is that isopentenyl pyrophosphate, the precursor for isopentenyladenosine, is generated via the mevalonate pathway, thus linking the cholesterol biosynthetic pathway with selenoprotein expression [35] (Fig. 43.1). Such an assumption is supported by earlier studies employing some unusual side-effects of statins which are widely used 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Prolonged treatment of hypercholesterolemic patients with these molecules was shown to be associated with an impaired synthesis of selenoproteins thus leading to excessive oxidative damage and possibly myopathy [36]. Importantly, it was demonstrated that treatment with statins directly impairs Gpx4 function [37]. These studies reconcile early findings showing increased muscular waste in animals treated with the statin, cerivastatin, by a mechanism that could directly be involved in muscle tissue repair that was recently suggested to be dependent on a Gpx4-mediated repair mechanism [38].

Another unique feature of selenoproteins that could be expanded to Gpx4 is the conversion of Sec to dehydroalanine (DHA). This oxidative modification is known to irreversibly inactivate the enzyme in a selenium-dependent manner. Despite the recognition that Cys residues can also be found in normal tissues in the form of Cys-SO<sub>2</sub>H [39], and some of them are converted to DHA [40], DHA is not frequently found in positions corresponding to Cys residues. This difference is likely attributable to the greater strength of the C-S bond (272 kJ/mol) compared to the

C–Se bond (234 kJ/mol). The specific characteristic of irreversible inactivation of Gpx4 may be relevant in conditions where the immune system may induce ferroptosis in malignant cells via the neutrophil oxidative burst by inactivating this selenoenzyme, thus again highlighting an important antitumoral role of selenoproteins and Gpx4.

### 43.3 In Vivo Relevance of Ferroptosis

At present, the in vivo implications of ferroptosis are scarce and its study is not trivial due to the absence of robust biomarkers. Currently, the implication and importance of ferroptosis in vivo can be inferred mostly based on genetic studies using models of putative regulators, namely xCT (SLC7A11),  $\gamma$ -GCS and Gpx4. Investigations using KO animals for targeting the aforementioned ferroptosis regulators have allowed us to draw important conclusions on which level these players act and when perturbed lead to engagement of this cell death modality. For instance, studies using xCT KO animals taught us that system  $x_c^-$  is dispensable for embryo development and KO animals are fully viable showing no overt phenotype [41], unless they are stressed by the redox cycling compound paraquat [42], or by ischemia/reperfusion injury in the kidney [43]. Therefore, it becomes obvious that conditions which modulate xCT activity or expression, at least in vivo and in non-transformed cells, cannot be regarded as an efficient pathological mechanism eliciting ferroptosis. This comes in contrast to the in vitro phenotype, whereby xCT inhibition efficiently triggers ferroptosis [20]. This is a phenomenon that is easily explained by the fact that under cell culture conditions Cys is only available in the form of cystine [22], which is in stark contrast to the in vivo situation [41]. Yet, xCT inhibition has been repeatedly proposed as a putative druggable target for inducing ferroptosis in tumors [24, 44]; therefore, caution should be taken when proposing that inhibition of xCT in vivo decreases tumor burden due to increased ferroptotic rates in cancer cells.

On the other hand, *Gpx4* KO animals provided the most valuable insights into which organs are susceptible to this form of cell death (Table 43.1). Specifically, the whole body KO of *Gpx4* is embryonic lethal at E7.5 [6], which is in agreement with the whole body KO of  $\gamma$ -GCS [45], thus supporting Gpx4 as the critical enzyme responsible for the pro-survival function of GSH. Recently, two studies independently provided definitive proof that the active site of Gpx4 (Sec) is indeed essential for early embryo development as mice homozygous for Sec-Ala or Sec-Ser mutations invariably died similar to systemic *Gpx4* KO mice [46, 47].

With the implementation of conditional KO for *Gpx4*, the specific study of the role of Gpx4 in particular tissues was made possible [28]. The first conditional KO of *Gpx4* was generated using the *CamKII $\alpha$ -Cre* mice, which deletes Gpx4 in neurons. These animals were born at normal Mendelian rates, but after 2 weeks following birth, they developed ataxia, seizures, neurodegeneration in the CA3 region of the hippocampus and premature death. These manifestations highlighted



**Table 43.1** Knockout (KO) mouse models for Gpx4

Year	Tissue (Cre line)	Major outcome	Ref.
2003	Systemic	Embryonic lethality at E7.5	[6, 57]
2005	Nuclear form ( <i>nGpx4<sup>-/-</sup></i> )	Fully viable, but impaired sperm chromatin condensation	[12]
2008	Ubiquitous (Cre-Deleter)	Embryonic lethality at E7.5	[28]
2008	Systemic	Early embryonic lethality	[58]
2008	Neurons ( <i>CamkII<math>\alpha</math>-Cre</i> )	Ataxia, seizures, neurodegeneration in CA3 region of hippocampus; premature death around 2 weeks after birth	[28]
2009	Mitochondrial form ( <i>mGpx4<sup>-/-</sup></i> )	Fully viable but exhibit male infertility due to malformation of mitochondrial capsules of sperm	[13]
2009	Overexpression of cytosolic form in <i>Gpx4<sup>-/-</sup></i> background	Fully viable but exhibit male infertility due to lack of mGpx4 expression	[11]
2009	Spermatocytes ( <i>Pgk2-Cre</i> )	Male infertility due to atrophy of germinal epithelium, morphologically aberrant, dysfunctional spermatozoa	[59]
2010	Neurons ( <i>CamkII<math>\alpha</math>-Cre</i> )	Seizures, ataxia and loss of parvalbumin-positive interneurons strongly resembling loss of <i>Trsp</i>	[48]
2012	Photoreceptor cells ( <i>Crx-Cre</i> )	Complete loss of photoreceptor cells by P21	[52]
2013	Endothelium ( <i>Cdh5(PAC)-CreER<sup>T2</sup></i> )	Vitamin E-deprived KO display many thromboembolic events causing various clinical pathologies, e.g., heart failure, renal and splenic micro-infarctions or paraplegia	[56]
2013	Inducible full body deletion ( <i>ROSA26-CreER<sup>T2</sup></i> )	Early death 2 weeks after KO induction; loss of neurons in the hippocampus	[51]
2013	Skin ( <i>K14-Cre</i> )	In perinatal mice, epidermal hyperplasia, dysmorphic hair follicles and alopecia	[53]
2014	Neurons ( <i>Tal1-Cre</i> )	Cerebellar hypoplasia and Purkinje cell death	[49]
2014	Inducible full body deletion w/o brain ( <i>ROSA26-CreER<sup>T2</sup></i> )	Renal tubular cell death, acute renal failure and death at ~12 days upon KO induction, which cannot be rescued by breeding on a 12/15-lipoxygenase ( <i>Alox15</i> ) KO background	[17]
2015	Motor neurons ( <i>Thy1-cre/ERT2,-EYFP; SLICK-H</i> )	Death of motor neurons and rapid onset and progression of paralysis and death	[50]
2015	T cells ( <i>CD4-Cre; ROSA26-CreER<sup>T2</sup></i> )	CD8+ T cells fail to maintain homeostatic balance in periphery; CD8+ and CD4+ T cells fail to expand and to protect mice from acute lymphocytic choriomeningitis virus and <i>Leishmania major</i> parasite infections	[55]
2015	Systemic (Sec-Ala mutation)	Early embryonic death, which cannot be rescued by breeding on an Alox15 KO background	[46]
2015	Systemic (Sec-Ser mutation)	Early embryonic death of homozygous mice, but impaired male fertility in heterozygous mice	[47]
2015	Hematopoiesis ( <i>ROSA26-CreER<sup>T2</sup></i> )	Anemia caused by receptor interacting protein kinase 3 (RIP3)-dependent necroptosis in erythroid precursor cells.	[54]



the importance of this cell death pathway for neuronal populations. Subsequently, studies using an alternative neuron-specific deletion strain, i.e., *Ta1-Cre*, have demonstrated that loss of Gpx4 in cerebellar neurons leads to cerebellar hypoplasia and Purkinje cell death [48, 49].

More recently, the first inducible KO for *Gpx4* in a neuronal population was reported using *Thy1-Cre/ERT2,-EYFP (SLICK-H)* mice [50]. Thereby, it was shown that the inducible deletion in adult animals leads to neurodegeneration manifested by the loss of motor neurons, thus underlining the importance of Gpx4 in preventing motor neuron degeneration. In an earlier study, the same authors have demonstrated that the inducible full body deletion (*ROSA26-CreER<sup>T2</sup>*) was accompanied by early lethality and loss of hippocampal neurons [51]. Nonetheless, it is unlikely that the lethality in this model is due to the loss of hippocampal neurons (see below). Other cells or organs that have been shown to be sensitive to Gpx4 deletion are photoreceptor cells [52], skin [53] and cells of the hematopoietic system [54].

Remarkably, none of the phenotypes observed above have critically interrogated the role of Gpx4 in the ferroptotic process. Therefore, an important contribution to the understanding of ferroptosis in vivo was recently provided. Using inducible full body deletion of Gpx4 except in the brain (*ROSA26-CreER<sup>T2</sup>*), we showed that early mortality of mice is due to acute kidney failure and this process is accompanied by an unrestrained and complex lipid oxidation signature [17]. Importantly, in this report, the first in vivo inhibitor of ferroptosis, liproxstatin-1, was described. This molecule was identified in a phenotypic small molecule screen for compounds able to inhibit cell death of mouse embryonic fibroblasts triggered by genetic deletion of *Gpx4*. Of note, liproxstatin-1 was able to prolong survival of *Gpx4* KO animals by preventing cell death in kidneys, thus providing first evidence that, at least in kidneys, Gpx4 deletion leads to cell death in a ferroptotic manner. Similar findings were also observed in T-cell-specific *Gpx4* KO mice [55], where upon deletion, CD8+ T cells not only failed to maintain homeostatic balance in periphery, but also CD8+ and CD4+ T cells failed to expand and to protect the mice from acute lymphocytic choriomeningitis virus and *Leishmania major* parasite infections. Hence, these observations support an important role of Gpx4 in restraining ferroptosis in immunity.

Another important lesson learned from the studies of *Gpx4* KO animals is that not all tissues respond in a similar manner as they may use ways to compensate for the loss of Gpx4. One of the mechanisms that appears to compensate for the loss of Gpx4 is the content of the antioxidant vitamin E in the diet. This notion was convincingly demonstrated using animals deficient for Gpx4 in the endothelium (*Cdh5(PAC)-CreER<sup>T2</sup>*) [56]. The endothelial-specific *Gpx4* KO animals developed normally and no marked phenotype or dysfunction in the endothelium was evident upon KO induction by tamoxifen, although aortic explants from endothelium-specific *Gpx4* KO presented a reduced number of endothelial branches in an ex vivo sprouting assay. From these findings, it was concluded that an unknown component was rescuing the loss-of Gpx4 in vivo. In order to challenge this, animals were then subjected to a diet with low levels of vitamin E, which in combination with the loss of Gpx4 led to several clinical pathologies, such as thromboembolic events

culminating in heart failure, renal and splenic micro-infarctions or paraplegia. Therefore, at least in the endothelium, an important interrelationship between Gpx4 and vitamin E must be considered, suggesting to some extent overlapping functions in protecting the endothelium against lipid peroxidation-driven dysfunction [56].

### 43.4 Concluding Remarks

It has become increasingly evident that cells can die in many ways which are not restricted to apoptosis. The recognition of novel cell death modalities has presented the cell death field with renewed breath, spurring research investigating the mechanisms regulating these new novel forms of cell death. Among these pathways, special attention has been attributed to ferroptosis due to its role in pathophysiological scenarios. Additionally, the recent discovery that this form of cell death is negatively regulated by the selenoprotein Gpx4 has opened new avenues of investigation in order to better understand the importance of Gpx4 and selenium biology in the context of tumor development and cytoprotective strategies. We believe that the integrated study of selenoprotein biology and ferroptosis will provide exciting future insights in the cross-talk between cell death and survival decisions in a myriad of pathophysiological phenomena.

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### References

1. R Brigelius-Flohe, M Maiorino 2013 *Biochim Biophys Acta* 1830:3289
2. L Flohe et al 2011 *Antioxid Redox Signal* 15:763
3. V Bosello-Travain et al 2013 *Biochim Biophys Acta* 1830:3846
4. M Conrad et al 2015 *Biochim Biophys Acta* 1850:1566
5. F Ursini et al 1982 *Biochim Biophys Acta* 710:197
6. LJ Yant et al 2003 *Free Radic Biol Med* 34:496
7. M Conrad 2012 in *Selenium: Its Molecular Biology and role in Human Health*, DL Hatfield et al Eds (Springer Science + Business Media, LLC, New York) p 547
8. H Pfeifer et al 2001 *FASEB J* 15:1236
9. TR Pushpa-Rekha et al 1995 *J Biol Chem* 270:26993
10. M Schneider et al 2006 *Gene Expr Patterns* 6:489
11. H Liang et al 2009 *J Biol Chem* 284:30836
12. M Conrad et al 2005 *Mol Cell Biol* 25:7637
13. M Schneider et al 2009 *FASEB J* 23:3233
14. M Conrad et al 2007 *Biol Chem* 388:1019
15. C Latchoumycandane et al 2012 *J Biol Chem* 287:17693
16. K Nomura et al 2000 *Biochem J* 351:183

17. JP Friedmann Angeli et al 2014 *Nat Cell Biol* 16:1180
18. T Van den Berghe et al 2014 *Nat Rev Mol Cell Biol* 15:135
19. M Conrad et al 2016 *Nat Rev Drug Discov* 15(5):348
20. SJ Dixon et al 2012 *Cell* 149:1060
21. WS Yang, BR Stockwell 2008 *Chem Biol* 15:234
22. S Bannai, E Kitamura 1980 *J Biol Chem* 255:2372
23. S Tan et al 2001 *Curr Top Med Chem* 1:497
24. L Jiang et al 2015 *Nature* 520:57
25. WS Yang et al 2014 *Cell* 156:317
26. H Imai et al 1998 *J Biol Chem* 273:1990
27. CJ Chen et al 2000 *Prostaglandins Leukot Essent Fatty Acids* 62:261
28. A Seiler et al 2008 *Cell Metab* 8:237
29. M Gao et al 2015 *Mol Cell* 59:298
30. T LaVaute et al 2001 *Nat Genet* 27:209
31. JC Ruiz et al 2013 *J Biol Chem* 288:552
32. SJ Dixon et al 2015 *ACS Chem Biol* 10:1604
33. N Fradejas et al 2013 *Biochem J* 450:427
34. GJ Warner et al 2000 *J Biol Chem* 275:28110
35. B Moosmann, C Behl 2004 *Trends Cardiovasc Med* 14:273
36. B Moosmann, C Behl 2004 *Lancet* 363:892
37. A Kromer, B Moosmann 2009 *Mol Pharmacol* 75:1421
38. M Labazi et al 2015 *Free Radic Biol Med* 84:246
39. M Hamann et al 2002 *Methods Enzymol* 348:146
40. J Seo et al 2008 *J Proteome Res* 7:587
41. H Sato et al 2005 *J Biol Chem* 280:37423
42. S Kobayashi et al 2012 *Free Radic Biol Med* 53:2197
43. T Shibasaki et al 2009 *Arch Biochem Biophys* 490:63
44. SJ Dixon et al 2014 *Elife* 3:e02523
45. ZZ Shi et al 2000 *Proc Natl Acad Sci U S A* 97:5101
46. SH Brutsch et al 2015 *Antioxid Redox Signal* 22:281
47. I Ingold et al 2015 *J Biol Chem* 290:14668
48. EK Wirth et al 2010 *FASEB J* 24:844
49. EK Wirth et al 2014 *Biol Trace Elem Res* 158:203
50. L Chen et al 2015 *J Biol Chem* 290:28097
51. SE Yoo et al 2012 *Free Radic Biol Med* 52:1820
52. T Ueta et al 2012 *J Biol Chem* 287:7675
53. A Sengupta et al 2013 *J Invest Dermatol* 133:1731
54. O Canli et al 2016 *Blood* 127:139
55. M Matsushita et al 2015 *J Exp Med* 212:555
56. M Wortmann et al 2013 *Circ Res* 113:408
57. H Imai et al 2003 *Biochem Biophys Res Commun* 305:278
58. MR Garry et al 2008 *Free Radic Biol Med* 44:1075
59. H Imai et al 2009 *J Biol Chem* 284:32522