Glutathione peroxidase 4: A new player in neurodegeneration?

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Running title: **Glutathione peroxidase 4 role in neurodegeneration**

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Abstract

The selenoprotein glutathione peroxidase 4 has been recognized for its antioxidant role, and recently has been reported as an important inhibitor of ferroptosis, a non-apoptotic form of cell death. Such death pathways were primarily described in cancer cells, but it has also been identified in hippocampus and renal cells. Here we link the role of this selenoprotein on ferroptosis with possible protective mechanisms of neurodegeneration. Additionally, we propose that selenium (Se) insufficient diet enhance the susceptibility of ferroptosis, as well as other death cell pathways, due to downregulation of GPx4 activity. We review recent findings on GPx4 with emphasis on neuronal protection, and associate the relevance of Se on its activity.

Introduction

Selenium (Se) is an essential nutrient required to synthesize selenocysteine (Sec), the 21st amino acid, which is incorporated into biomolecules by translational coding during selenoprotein synthesis. Twenty-five different selenoproteins have thus far been identified in human proteome[1]. Among them, the glutathione peroxidase (GPx) family, compound by 8 sequentially numbered isoenzymes that catalyze the reduction of H_2O_2 of organic hydroperoxides by glutathione (GSH) or other biological reductants. Although they are all in the same family, each enzyme has various characteristics that determine their biological role (Table 1). Only GPx1, 2, 3 and 4 are considered selenoproteins in all mammals, as they incorporate Sec as part of catalytic site; GPx6 is considered a selenoprotein in humans though not in rodents; and GPx5, 7 and 8 use cysteine (Cys) in place of Sec[2].

In the brain, GPx enzymes are expressed in neurons and glial cells[3, 4], where their free radicals scavenging role protects against oxidative stress. GPx4 is the most widely expressed isoform in brain, existing as a membrane anchored glycoprotein[5] that functions to reduce a wide range of complex hydroperoxy lipids, and also accepts various thiols as reductants[6]. GPx4 was recently recognized as a key regulatory factor in ferroptosis, a newly discovered non-apoptotic programmed cell death pathway characterized by iron-dependent metabolic dysfunction that causes a rapid elevation in the levels of reactive oxygen species[7]. Further, the GPx4 is essential for development and cell survival; evidenced in animal models that had Sec replaced by serine in the GPx4 catalytic site[8]. As the function of the GPx family is key to normal development and cellular metabolism *via* the regulation of oxidative stress, GPx4 dysfunction is a potential Achilles' heel for cell survival.

In this Perspective, we discuss the function of GPx4 in the brain, and suggest this enzyme may be a key regulator of neurodegeneration. In doing so, we also examine the essential function of GPx4 and the association of Se nutritional status and supplementation, describing the potential benefits on neuronal maintenance *via* promoting GPx4 activity and expression.

Biological function of GPx4

GPx4, as well as the other Se-containing GPx enzymes, is recognized by its antioxidant role, and has the catalytic center characterized by a tetrad comprising Sec hydrogen-bonded to the nitrogen of asparagine (Asn), glutamine (Gln) and tryptophan (Trp) residues[9]. Four different GPx4 were identified: cytosolic and mitochondrial GPx4, both coded by all 7 exons; sperm nuclear GPx4, which is encoded by an alternative exon in

the first intron[10, 11]; and GPx4-I, which was recently detected in immortalized mouse hippocampal neuron cells (HT22) and is coded by the intron sequence between exons 1b and 2[12]. Cytosolic-GPx4 is ubiquitous in cells and the main isoform in neurons. Its activity has been either observed in both membrane and soluble compartments[13]. Mitochondrial and sperm nuclear GPx4 are predominantly expressed in testes, but also found in neurons.

All GPx4 isoforms are distinct from other members of the GPx family, as it exists as a monomer. The ability of GPx4 to reduce complex hydroperoxy phospholipids and cholesterol is partially due to absence of an internal sequence of 20 amino acids forming a surface-exposed loop that regulates substrate specificity in other GPx molecules[10, 14]. The decreased substrate specificity also supports GPx4 having a wider range of reducing substrates that allow it to function when GSH levels are low. In diseases where high production of reactive oxygen and nitrogen species (ROS/RNS) coincides with low GSH levels, as observed in some psychiatric disorders[15] and neurodegenerative diseases[16], the low specificity of GPx4 for reducing substrates is likely to contribute to the diverse maintenance roles it has in neurons.

GPx4 reaction kinetics share similarities with GPx1 and 3; characterized by three steps following a 'ping-pong' mechanism, where bimolecular reactions between the enzymes and substrate sequentially comprise catalytic cycles[9]. As the reaction mechanism involves the oxidation of Se by hydroperoxide without the formation of any enzyme–substrate complex, the enzyme is never completely reduced *in vivo*, and thus the reaction rate depends on the concentration of GPx4 and hydroperoxides, and not on the concentration of GSH. This implies a relevant difference between the physiological process and the conditions *in vitro*, because under controlled conditions, hydroperoxide and GSH concentrations are close to equimolar and reactions tend to be more dependent

on GSH levels[2]. Under conditions of hydrogen peroxide signaling, the membrane anchored form of GPx4 would be particularly susceptible to over-oxidation to selenic acid[22], thus allowing signaling to occur consistent with the 'flood-gate' model of signaling[23].

Ablation of *GPx4* or expression of enzymatically inactive GPx4 is embryonically lethal[8, 24], and thus only conditional knockout or heterozygous mice can be studied. Mitochondrial *GPx4* null mice are available, although males are infertile due to abnormalities in sperm maturation[25, 26]. Sperm nuclear GPx4 is required for the structural integrity of mammalian sperm chromatin[27], and cytosolic-GPx4 is essential for embryonic survival and development, as may compensate the antioxidant and anti-apoptotic role in the absence of the other isoforms. It is suggested that cytosolic-GPx4 can also be found in the mitochondrial intermembrane and in the nucleous[28, 29]. GPx4 is predominantly expressed in developing brain, and neuronal *GPx4* null mice are not viable[30]. Conditional knockout of GPx4 in developed mice at 6 to 9 months of age exhibit hippocampal neurodegeneration, indicating the necessity of GPx4 for brain development and maintenance[31].

GPx4 and oxidative stress in the brain

Increased markers of oxidative stress have been identified in Alzheimer's disease[32-34], Parkinson's disease[35, 36], multiple sclerosis[37, 38] and amyotrophic lateral sclerosis[39, 40]. The brain has particular characteristics that result in increased vulnerability to oxidative stress. It has the highest metabolic activity compared to any other tissue, as it requires constant production of large amounts of ATP and resultant byproducts of mitochondrial function to maintain neuronal homeostasis. The brain accounts for only 2% of the total

116 body mass, yet consumes 25% of its energy[41]. All brain cells produce high levels of
117 nitric oxide (NO) for signal transduction, which amplifies the potential for peroxynitrite
118 formation. Further, neuronal plasma membranes are rich in polyunsaturated fatty acids
119 (PUFAs) that are particularly vulnerable to free radical attack and peroxidation of
120 unsaturated carbon-carbon bonds.

121 In the brain, antioxidant mechanisms exist in a synergy between small molecular weight
122 antioxidants (*e.g*. ascorbate, and vitamin E) to directly neutralize ROS and RNS; and
123 enzymatic systems comprised of catalase, superoxide dismutase and the glutathione
124 peroxidases. GPx4 is synthesized endogenously in the brain (Figure 1) found
125 predominantly in neurons of the cerebellum, hippocampus and hypothalamus[6].
126 However, following brain injury, this selenoprotein is upregulated in reactive astrocytes
127 of damaged areas, indicating protective role counteracting cellular deterioration
128 throughout the brain[42]. Under conditions of brain injury or neurodegeneration key lipid
129 biomarkers of nitrative and oxidative stress are elevated including the nitrotyrosine,
130 nitro-tocopherol and lipid oxidation products (*e.g*. malondialdehyde, acrolein and 4-
131 hydroxynonenal)[43-46]. The key role of the lipid oxidation products in ferroptosis and the
132 role of GPx4 in detoxification indicate the crucial role of Sec in GPx4 healthy cell
133 maintenance.

134 GPx4 has anti-apoptotic role due its capacity to inhibit peroxidation of cardiolipin.
135 Because cytochrome-*c* only binds to cardiolipin (CL), but not to its hydroperoxide state
136 (CL-OOH), the protection of cardiolipin suppress the release of cytochrome-*c* from
137 mitochondria[47] (Figure 2b). GPx4 also modulates ATP generation under oxidative
138 conditions[48], which has potential implications regarding mitochondrial dysfunction in
139 Alzheimer's[49] and Parkinson's diseases[50]. However, due to the high levels of PUFAs in
140 neurons, peroxidation is perhaps the most relevant oxidative stress process associated

141 with neurodegeneration. Recently, Seiler *et al*.[30] described an apoptotic mechanism
142 induced by lipid peroxidation resulting from 12/15-lipoxy-genase activities, and
143 highlighted the importance of this pathway in neuronal cells. This process results in
144 translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus,
145 leading to large-scale DNA fragmentation and cell death (Figure 2a). In the brain, ROS
146 and RNS are released constantly through neurotransmission and mitochondrial activity.
147 Physiologically, these molecules are either reduced spontaneously in the cytoplasm, or
148 enzymatically processed by superoxide dismutase, resulting in the production of
149 hydrogen peroxide (H_2O_2), which easily permeates cell membranes if not neutralised by
150 GPx or catalase. Hydrogen peroxide also reacts with redox-active copper or iron *via* the
151 Fenton/Heiber Weiss reaction, and is converted to a hydroxide anion and hydroxyl
152 radical that favours the formation of lipid peroxides (-LOOH). Lipid peroxidation is a
153 particularly damaging cycle, as these reaction products also act as triggers for the
154 generation of additional lipid peroxides in the membrane *via* lipoxygenases that catalyze
155 the oxygenation of polyunsaturated fatty acyl groups to hydroperoxides. In this
156 scenario, 12/15-lipoxygenase is relevant because only a low amount of peroxide is
157 needed and it can oxidize complex lipid esters even when incorporated in membranes or
158 lipoproteins[51]. Interestingly, it has been shown that GPx4 ablation results in propagation
159 of lipoperoxydation cascade *via* activation of 12/15-lipoxygenase, and treatment with α-
160 tocopherol efficiently prevented this apoptotic response activated by GPx4 deficiency.

161 Reactive nitrogen species have a prominent role in the pathology of neurodegenerative
162 diseases. In Alzheimer's disease, Parkinson's disease and motor neuron disease 3-
163 nitrotyrosine is a biomarker of neurodegeneration, as are protein carbonyls that can
164 result from peroxynitrite-induced oxidation of proteins. The CNS produces
165 approximately 20 times more NO than the cardiovascular system. Nitric oxide is a

166 critical secondary messenger, however in the presence of superoxide it will react at
167 diffusion limited rates to produce peroxynitrite[52], making it likely to be a key oxidant in
168 the brain (Figure 2c). Selenium containing compounds have a 250-800 fold faster
169 reactivity with peroxynitrite than corresponding thiol containing compounds[53]. GPx
170 enzymes and the lipophilic selenium-containing molecule Ebselen (2-Phenyl-1,2-
171 benzoselenazol-3-one) are protective against peroxynitrite. Ebselen is a biomimetic of
172 GPx function and has protective effects against lipid peroxidation[54, 55]. The increased
173 electrophilicity of Sec compared to Cys and the decreased pK_a of Sec indicate that
174 GPx4 is a key defense enzyme to protect against peroxynitrite induced lipid
175 peroxidation. Thus, a key function of GPx4 and potentially other selenoenzymes in the
176 CNS could be the protection of the cell from peroxynitrite. In addition to GPx4 being a
177 scavenger of organoperoxides it is also a peroxynitrite reductase[56, 57]. The reaction of
178 peroxynitrite with GPx has been calculated to be a more efficient substrate for GPx than
179 hydrogen peroxide[58].

180

181 **Selenium: key element for GPx4 activity in neurodegeneration**

182 *In vivo* studies have shown that Cys can replace Sec in different selenoproteins, as these
183 analogous amino acids differ only in the substitution of selenol moiety by a thiol
184 group[59, 60]. In the same way, GPx isoenzymes, including GPx4, have Cys homologues[61],
185 however the presence of Sec confers increased activity to the Se-containing GPx
186 enzymes[59, 60, 62]. Indeed, studies designed in an *Escherichia coli* expression system
187 showed that a recombinant Cys mutant of GPx4 had a significant depletion of catalytic
188 efficiency and 1000-fold lower activity compared to the natural enzyme[63-65]. This is due
189 to the more acidic pK_a of Sec ($pK_a = 5.5$)[66-68] compared to Cys ($pK_a = 8.3$). The Sec
190 provides an alternative solution to controlling the pK_a of the reactive site residues than

191 modification of the local structure of the protein. Concurrently, the local structure can
192 also influence the pK_a of Sec in proteins further[69], as occurs in a dramatic shift in the
193 pK_a of Cys that can be as low as ~3 for thiol:disulfide interchange proteins DsbA[70, 71].
194 Mannes *et al*.[72] showed that, at a physiological levels, Cys-GPx4 prevented death of
195 murine embryonic fibroblast cells in GPx4 knockout mice *via* an anti-apoptotic
196 mechanism. However, to obtain a comparable function to natural GPx4, more Cys
197 mutant was required. Incorporation of Cys instead of Sec in selenoproteins is dependent
198 of Se availability to the cells[60], thus Se deficiency directly impacts on GPx4 production
199 and activity in the brain. Studies have shown the positive effects of Se supplementation
200 in recovering GPx4 activity. Sodium selenite (Na_2SeO_3) treatment restored GPx1 and
201 GPx4 activity in oxidatively stressed methamphetamine-treated SH-SY5Y cells[73].
202 Similarly, mice with induced-neurotoxicity by patulin had increased mRNA levels of
203 GPx1 and 4 after treatment with selenomethionine[74].

204 The advantage of Sec compared to Cys is important in the central nervous system, as
205 pH in synaptic vesicles varies constantly. Synaptic transmission causes strong
206 acidification in the synaptic cleft due to release of protons, which is subsequently
207 followed by increase in extrasynaptic pH[75]. Thus, under acidic pH, Sec would be
208 deprotonated more quickly while thiol would still exist as -SH. Considering that GPx4
209 responds to Se supplementation, we hypothesize that Se supplementation might improve
210 GPx4 activity in different tissues by increasing the Sec to Cys ratio. Indeed, deficient Se
211 status in humans have been associated with risk for Alzheimer's and Parkinson's
212 diseases[76-79] and the supplementation with a natural source of highly bioavailable
213 selenomethionine improved cognition in mild cognitively impairment patients[80]. In
214 general, Se status is positively correlated with total GPx activity when measured in the
215 same compartment[81]. However, some questions arise when total GPx activity is used in

order to assess GPx4 function, as: i) there is no known correlation between total peripheral GPx activity and GPx4 in brain, as the isoenzyme GPx3 and GPx1 are the most abundant variants in plasma and erythrocytes, respectively[82, 83]; ii) circulating GPx4 is low, which makes assessment with adequate sensitivity and specificity difficult; and iii) total plasma GPx activity reaches a plateau when whole blood Se levels reach 1.3 μmol/L[84]. It is unknown what Se concentration is required for GPx4 activity to reach a plateau in the brain, and both *in vitro* and *in vivo* studies will help to elucidate the best Se dietary intake strategy that may contribute to increasing GPx4 activity in the brain as a means to intervene in neurodegenerative diseases progression.

Selenoprotein synthesis is modulated by refined mechanisms that control gene transcription, RNA processing, translation and also post-translational steps of protein biosynthesis. Both selenoprotein synthesis and the hierarchical mechanisms that distribute Se among tissues are tightly regulated, and it is believed that during periods of Se deficiency these mechanisms prioritize synthesis most important selenoproteins and distribution to organs with the highest need[85, 86]. *In vivo* studies show that brain, reproductive and endocrine organs have the highest priority for Se uptake and retention during Se deficiency[87-89]. Although levels of Se in the brain are low (~0.03 μg g^{-1} wet tissue) compared to other organs, the importance of Se in normal neural function has been demonstrated in studies where competition between high priority organs has been manipulated[89]. We postulate that dietary insufficiency of Se or impaired transport to the brain contributes to a decreased capacity of neurons to cope with the oxidative and nitrative stress, depleting an individual's resilience to developing neurodegenerative disease.

GPx4 and ferroptosis in neurodegeneration

Ferroptosis is characterized by metabolic dysfunction that causes increased production of reactive species of oxygen *via* an iron-dependent mechanism[7, 90]. In its first step, cysteine/glutamate antiporter system x_c^- is inhibited, and thus GSH biosynthesis is reduced (Figure 2d). As a consequence, GPx4 activity is negatively affected, resulting in increased lipid peroxidation[91, 92]. Although lipid peroxidation probably initiates outside the mitochondria independently of 12/15 lipoxygenase, oxidized mitochondrial phospholipids demonstrate effects within this organelle. Proteomic analysis has suggested that GPx4 is the sole member of the GPx family playing a central role as regulator of ferroptosis[92]. However, it remains unclear if other isoforms have an as-yet undiscovered contribution, and thus additional research on other members of the GPx family is needed to elucidate their involvement in this important new mechanism of programmed cell death.

Ferroptosis has been identified in cancerous[7, 92] and hippocampal cells[7]; and it has also been described as a trigger of acute renal failure[91]. Recently, Chen *et al*[93]. reported the participation of this mechanism in neurodegeneration. Adult (3-4 months of age) GPx4 neuronal inducible knockout transgenic mice treated with tamoxifen for *GPx4* ablation presented a striking paralysis phenotype. Interestingly, only cerebral cortex and hippocampal cells were not sensitive to reduced GPx4 activity, and so it remains unclear why different neuronal cells are disposed to ferroptosis, and if different forms of stress specifically activate ferroptosis in determined cells, such as elevated brain iron. Consistent with the importance of lipid peroxidation driving ferroptosis, alpha-tocopherol was protective and we therefore hypothesis that Ebselen would provide protection as well.

In light of these data reinforcing the relevance of GPx4 in neuronal health, it is important to better understand the molecular basis of this selenoenzyme in order to

optimize its activity as possible strategy for addressing neurodegeneration. Moreover, it is still unclear what effects Se treatment may have in reducing ferroptosis, and future studies should consider the bioavailability of different Se compounds. For instance, it is known that organic Se, as selenomethionine, has high availability and low toxicity, as can non-specifically substitute methionine in serum proteins, especially albumin[83]. In contrast, inorganic forms as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) have lower bioavailability and higher toxicity (reviewed by Thiry *et al*.[94]). Increased understanding of the biochemical role of Se in ferroptosis could provide novel pathways for targeted drug development to treat disease where ferroptosis is a key mechanism.

Modulating the role of GPx4 as a neuroprotective agent

The antioxidant role of GPx4 can be potentiated by association with other biologically active molecules, and this should be considered with regard to strategies designed to minimize neurodegeneration. For instance, *N*-acetylcysteine (NAC), a Cys-donor and biosynthetic, acts as precursor to GSH and was proven to prevent cell death from eracin-induced ferroptosis *in vitro*[92] (Figure 2d). Other studies have showed NAC has antioxidant activity[95, 96], and further experiments using physiological conditions are necessary to demonstrate a potential interaction of NAC with GPx4 in prevention of ferroptosis.

Docosahexaenoic acid (DHA) (22:6n-3) is the most abundant n-3 long-chain PUFA in the brain and has indirect antioxidant role associated with regulation of *GPX4* gene expression. Hippocampal HT22 cells treated with DHA showed increased expression of *GPX4* by around 50% after 48 hours. This regulation appeared to be exclusive to *GPX4*, as the isoenzyme 1 gene was not affected and no changes in its activity were observed[12].

On the other hand, a low-DHA diet also led to the stimulation of expression of all GPx4 isoforms in wild type animals, which suggests the occurrence of a compensatory genetic strategy to protect cellular membrane from peroxidation under DHA deficiency[97]. Other mechanisms by which DHA can act as a beneficiary to brain GPx4 activity have been described before, but these data specifically reinforce the associated mechanisms of different antioxidants and presents new avenues for optimizing ferroptosis inhibition as a viable therapeutic strategy.

Vitamin E (namely α-tocopherol, the most abundant isoform) is a potent antioxidant and is associated with GPx4 *via* a chain-breaking electron donor mechanism. In the brain, this micronutrient is at a low concentration, though the radical quenching reaction is extremely fast (~ 10^8 M^{-1} s^{-1})[98]. Alpha-tocopherol inhibits ferroptosis *in vitro*[7], and GPx4 neuronal inducible knockout transgenic mice treated with a vitamin E enriched diet showed a delayed paralysis phenotype linked to ferroptosis[93]. However, it is worth mentioning that vitamin E is dependent on the reduction of vitamin C, and so excessive supplementation might have a counterproductive pro-oxidant effect and induce ferroptosis. We hypothesize that under physiological levels, DHA and vitamin E availability to neuronal cells may be important regulators of ferroptosis by influencing GPx4 levels and activity in the brain (Figure 2d), and suggest that the nutritional status of these particular nutrients should be considered when interventions are made in order to optimize GPx4 activity as a strategy to inhibit neurodegeneration. Thus the nutritional status of vitamin E, of which deficiency is widely prevalent[99], and Se may be key for optimal health and resilience against oxidatively driven activation of ferroptosis, particularly in neurodegenerative diseases.

Conclusions

Se deficiency has been linked to increased oxidative stress and neurodegenerative diseases. However, different mechanisms may be intrinsic and here we propose that ferroptosis is another path by which Se has an important role in the maintenance of a healthy brain. Selenium is key factor for GPx4 expression and activity, and in deplete situation, selenoproteins present reduced activity due incorporation of Cys instead of Sec, which has negative implications for GPx4 activity and may increase susceptibility of the cell to oxidative stress and induction of ferroptosis.

We claim for further studies focused on elucidating the role of Se in both this newly-discovered mechanism of cell death, as well the possible association with other small molecules, such as NAC, DHA and α-tocopherol in order to establish new therapeutic strategies to prevent and delay diseases that affect millions of the people worldwide. We believe that optimization of nutritional status of Se may result in higher GPx4 activity and thus delay, or even prevent, neuronal loss. Increasing Se levels is likely to only contribute to a decreased risk in development of neurodegenerative disease in populations that have a decreased Se exposure. Understanding the role of Se proteins, oxidative stress and ferroptosis in neurodegeneration may provide a unique insight to the cellular death mechanisms that occurs in neurodegeneration.

Figure 1: Mechanism of blood-brain barrier transit of selenoprotein P (SelP) and resultant effects on brain selenoprotein synthesis. Selenium delivery into brain is dependent on selenoprotein P (SelP), which is endocytosed by apolipoprotein E receptor-2 (ApoER2) at the blood-brain barrier and releases Se into the interstitum. Astrocytes then resynthesize SelP to raise a pool of Sec available to the brain as required. ApoER2 is also expressed in neurons, and is the likely neuronal import route for SelP. GPx4 is synthesized endogenously in neurons, obtaining the necessary Se

from SelP transit across the neuronal membrane *via* the ApoER2, which increases the intracellular Sec:Cys ratio and stimulates transcription of a range of selenoproteins, including GPx4[100].

Figure 2: Glutathione peroxidase 4 modulates different pathways to inhibit neuronal loss. 2a. GPx4 reduces activation of 12/15-lipoxy-genase, inhibiting the translocation of AIF from the mitochondria to the nucleus, which leads to large-scale DNA fragmentation and cell death; 2b. In mitochondria, GPx4 inhibit the peroxidation of cardiolipin (CL) and then suppress the release of cytochrome-*c* from mitochondria and apoptosis signalling; 2c. GPx4 acts as scavenger of organoperoxide and peroxynitrite; 2d. Ferroptosis is characterized by the inhibition of the x_c^- system, responsible for Cys import, causing limited GSH biosynthesis. As GPx4 can reduce lipid peroxides when GSH levels are low, it is a negative regulator of this cell death pathway. Alpha-tocopherol, in a chain-breaking electron donor mechanism, plays antioxidant role in association with vitamin C, and thus is also considered negative regulator of ferroptosis. NAC is a GSH precursor because donates Cys. DHA upregulates GPx4 expression. Abbreviations: AIF: apoptosis-inducing factor; CL: cardiolipin; CL-OOH: cardiolipin hydroperoxide; Cys: cysteine; DHA: Docosahexaenoic acid; GPx4: glutathione peroxidase 4; GSH: glutathione; GSSH: glutathione disulfide; SOD1: superoxide dismutase 1; H_2O_2: hydrogen peroxide; H_2O: water; $LOO^{\cdot}$: lipid peroxide; NAC: *N*-acetylcysteine; NO: nitric oxide; $NO_2^{\cdot}$: nitrogen dioxide; $^{\cdot}O_2^-$: superoxide; $ONOO^-$: peroxynitrite; RNS: reactive nitrogen species; ROS: reactive oxygen species.

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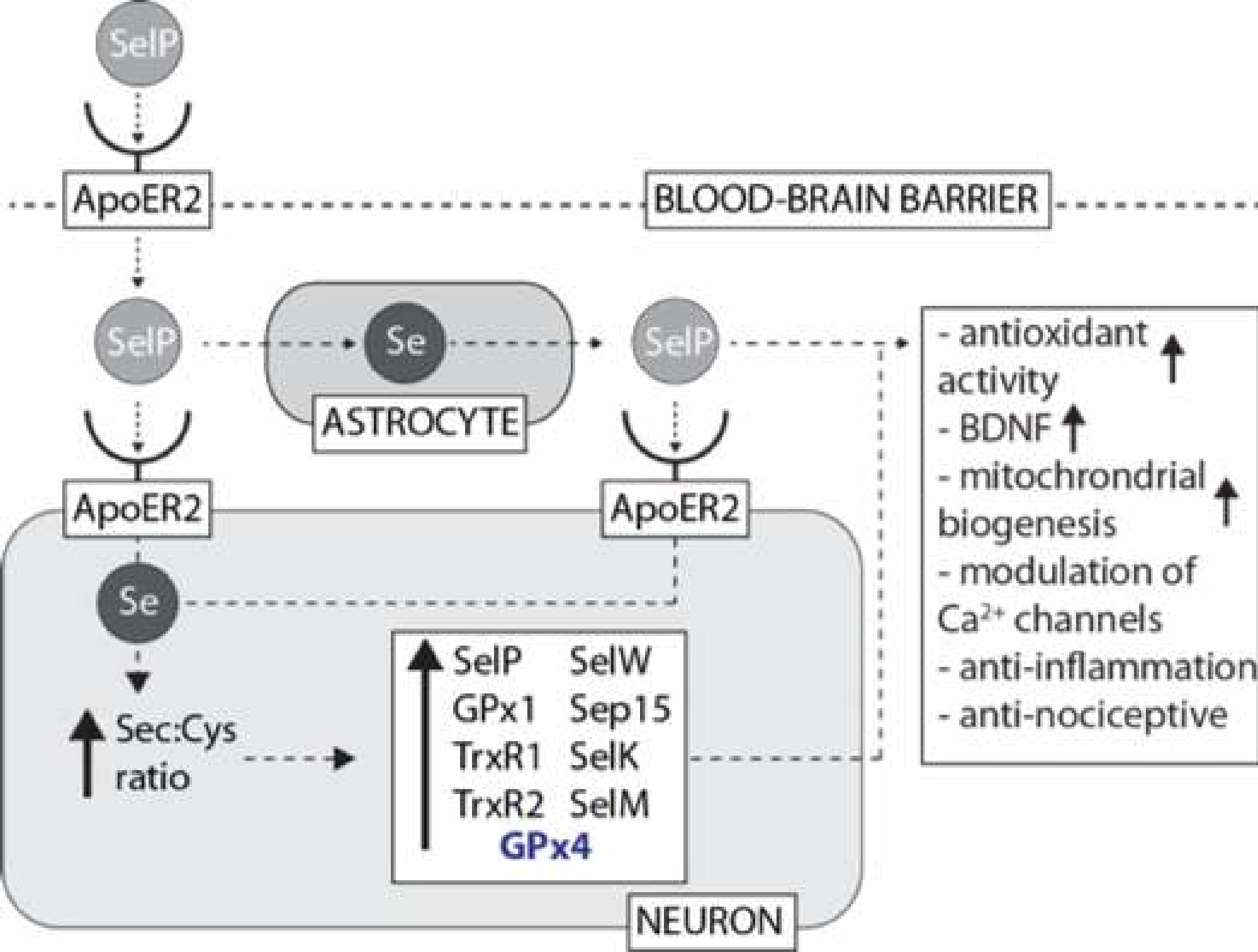
Table 1: Characteristics of mammalian glutathione peroxidases.

GPx type	Peroxidatic residue	Quaternary structure	Molecular weight (kDa)	Reducing substrate	Subcelullar location	Principal location
GPx1	Sec	tetramer	88.4 (isoform 1) 10.3 (isoform 2)	GSH	Cytoplasm, cytoson, mitochondrion	Kidneys, liver, erythrocytes
GPx2	Sec	tetramer	87.9	GSH	Cytoplasm	Gastrintestinal mucosa
GPx3	Sec	tetramer	102.2	GSH, thioredoxin, glutaredoxin	Extracellular	Plasma, kidneys, intestinal villus, adipose tissue, extracellular body fluids
GPx4	Sec	monomer	19.5 (cytosolic) 22.2 (mitochondrial)	GSH, cysteine, protein thiols	Cytoplasm, mitochondrion, nuleus	Testes, spermatozoa, brain
GPx5	Cys	tetramer	100.8 (isoform 1) 45.7 (isoform 2)	NA	Extracellular, plasma, membrane	Epididymis, spermatozoa
GPx6	Sec in humans Cys in mice	tetramer	99.9 (humans)	GSH	Secreted	Olfactory ephitelium
GPx7	Cys	monomer	21.9	GSH, protein disulfide isomerase	Secreted	-
GPx8	Cys	-	-	GSH	Cytoplasm	-

Cys: cysteine; GPx: glutathione peroxidase; GSH: glutathione; Sec: selenocysteine[1 2 3, 4 5].


SelP
ApoER2
BLOOD-BRAIN BARRIER
SelP
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ASTROCYTE
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ApoER2
ApoER2
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Sec:Cys ratio
SelP SelW
GPx1 Sep15
TrxR1 SelK
TrxR2 SelM
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NEURON
- antioxidant activity
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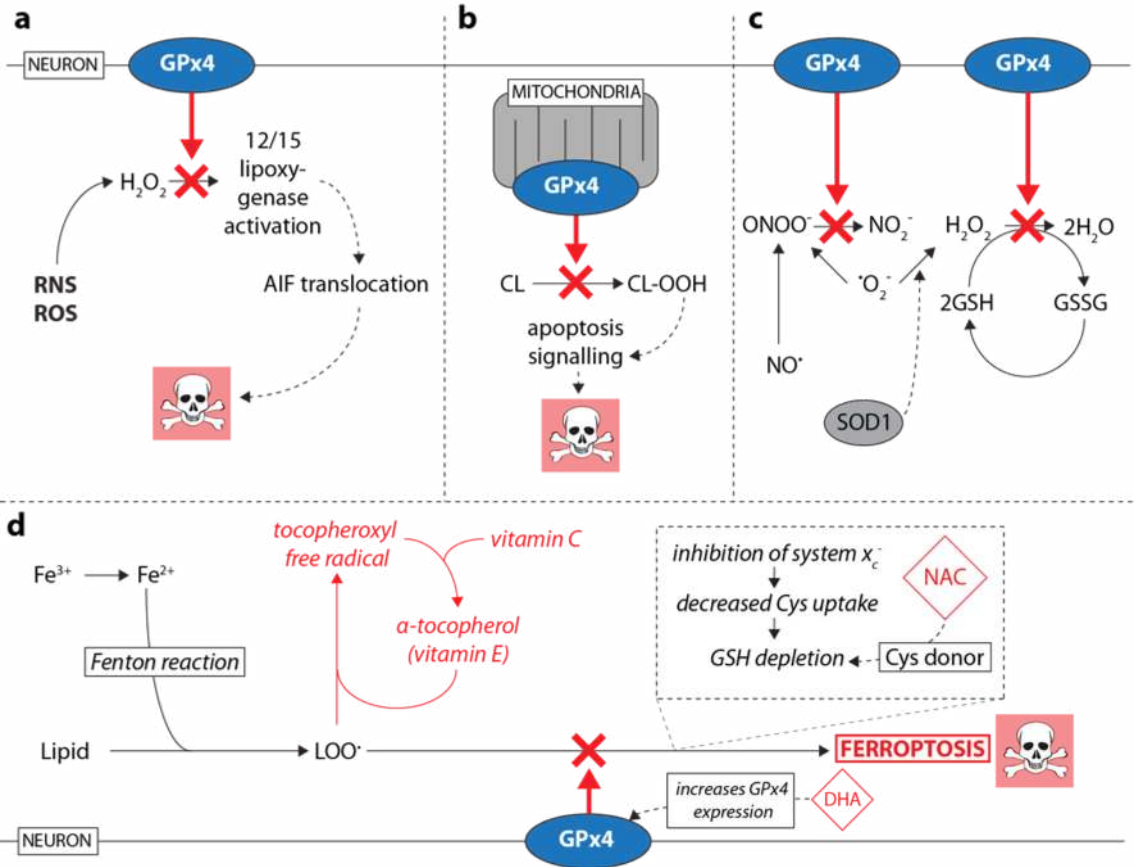
- mitochrondrial biogenesis
- modulation of Ca^{2+} channels
- anti-inflammation
- anti-nociceptive


a
NEURON
GPx4
H_2O_2
12/15 lipoxy-genase activation
RNS
ROS
AIF translocation
b
MITOCHONDRIA
GPx4
CL
CL-OOH
apoptosis signalling
c
GPx4
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NO_2^-
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NO•
SOD1
H_2O_2
$2H_2O$
2GSH
GSSG
d
Fe^{3+}
Fe^{2+}
Fenton reaction
tocopheroxyl free radical
vitamin C
α-tocopherol (vitamin E)
Lipid
LOO•
inhibition of system x_c^-
decreased Cys uptake
GSH depletion
NAC
Cys donor
FERROPTOSIS
increases GPx4 expression
DHA
GPx4
NEURON