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Review Article

NPGPx (GPx7): a novel oxidative stress sensor/transmitter with multiple roles in redox homeostasis

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Abstract: NPGPx (GPx7) is a member of the glutathione peroxidase (GPx) family without any GPx activity. GPx7 displays a unique function which serves as a stress sensor/transmitter to transfer the signal to its interacting proteins by shuttling disulfide bonds in response to various stresses. In this review, we focus on the exceptional structural and biochemical features of GPx7 compared to other 7 family members and described how GPx7 regulates the diverse signaling targets including GRP78, PDI, CPEB2, and XRN2, and their different roles in unfolded protein response, oxidative stress, and non-targeting siRNA stress response, respectively. The phenotypes associated with GPx7 deficiency in mouse or human including ROS accumulations, highly elevated cancer incidences, auto-immune disorders, and obesity are also revealed in this paper. Finally, we compare GPx8 with GPx7, which shares the highest structural similarity but different biological roles in stress response. These insights have thus provided a more comprehensive understanding of the role of GPx7 in the maintenance of redox homeostasis.

Keywords: GPx, stress, cysteine, ROS, redox, GPx7-interactiong protein

Introduction

Oxidative stress is generated when there is an imbalance between systemic manifestation of reactive oxygen species (ROS) and the biological detoxification processes. ROS are reactive oxygen-containing molecules that include oxygen ions and peroxide (R-O-O-H or R-O-O-R). ROS are produced as natural products from multiple intrinsically means, such as growth factor signaling in cytoplasm [1], oxidative respiration in mitochondria, and oxidative protein folding in endoplasmic reticulum [2], respectively. ROS can also be induced by external stimuli such as UV-irradiation [3] and viral infections [4]. The intrinsic level of ROS plays a critical role in maintaining cellular homeostasis [5]. For example, neutralization of ROS by N-acetylcysteine (NAC) in preadipocytes reduces the adipogenesis efficiency [6]. Persistent exposure to oxidative stress leads to systemic abnormalities, such as autoimmunity [7], cardiovascular dysfunction, carcinogenesis [8], diabetes mellitus, obesity [9], neurodegeneration [10], and aging [11]. Moreover, recent studies suggested that oxidative stress may induce depression, and associate with the decrease of neuronal and glial cells in cortical-limbic regions [12]. In this context, sensing and releasing the excessive ROS is crucial in maintaining physiological homeostasis.

The living organisms have developed multiple systems to defend oxidative stress. Catalase (CAT), peroxiredoxin (PRDX), and glutathione peroxidases (GPxs) are three of the most important enzyme families that involve in ROS-removing process. CAT directly decomposes hydrogen peroxide (H_2O_2) to water in peroxisome. PRDX catalyzes the reduction of H_2O_2 to water coupled with thioredoxin oxidation [13]. The third group, GPx family, involves in the maintenance of H_2O_2 homeostasis through their GPx enzyme activity using glutathione (GSH) as a reducing substrate. Up to date, based on their structural similarities, eight GPxs have been

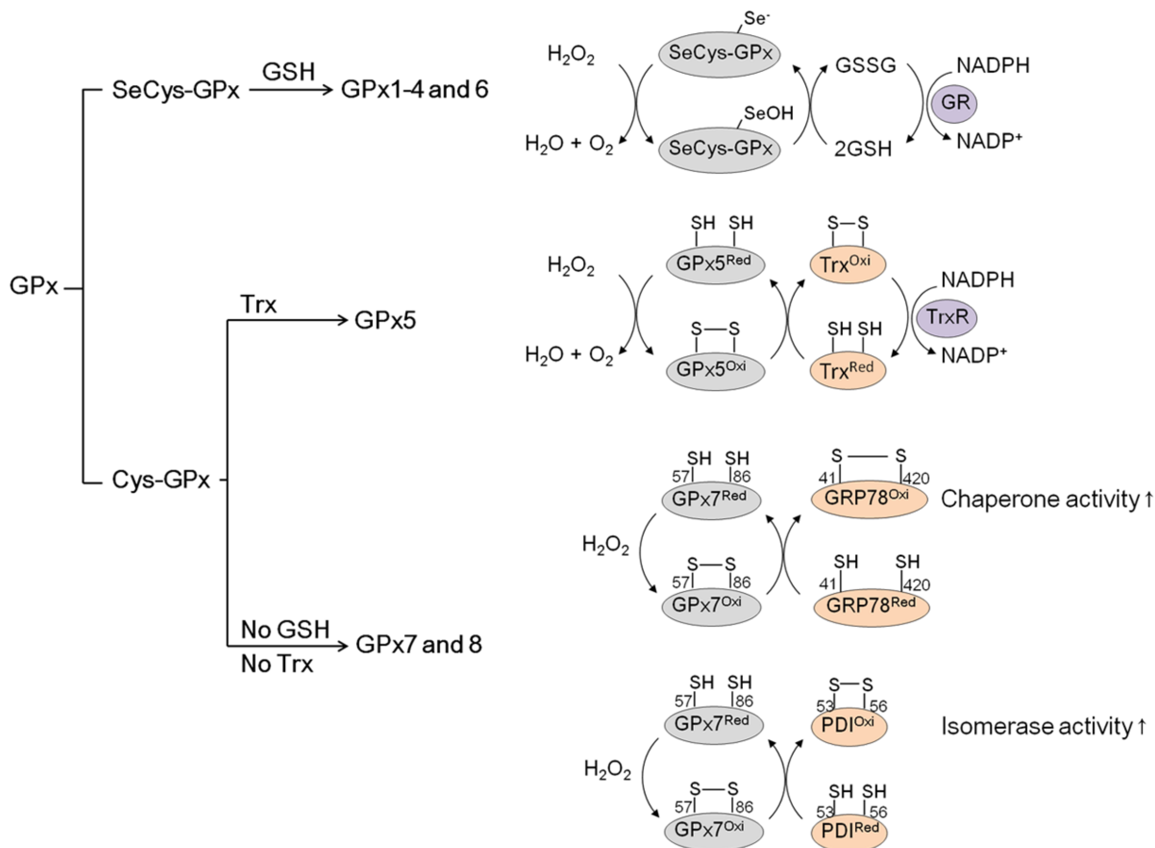

Figure 1. Classification and redox reaction of GPxs. GPx1-4 and 6 catalyze H_2O_2 reduction using GSH or thioredoxin (Trx) as cofactors. GPx7 modifies cysteine residues in response to oxidative stress that is coupled with disulfide shuttling with its interacting proteins, such as GRP78 and PDI, respectively. The change of thiol moiety (-SH) state of cysteines alters chaperone or enzyme activities [86]. For GPx8 activities please see text for details [84]. Abbreviations: GR, glutathione reductase; TrxR, thioredoxin reductase.

identified in mammalian cells [14-17]. They are grouped as selenocysteine containing GPx (GPx1-4, GPx6) and non-selenocysteine containing GPx (GPx5, GPx7 & 8), with the later incorporates the cysteine residue instead of the selenocysteine in the protein (**Figure 1**) [17]. Selenocysteine-containing GPxs catalyze the conversion of H_2O_2 to water (H_2O) and oxygen (O_2) by coupling with glutathione (GSH) oxidation. GSH binding specificity is determined by three factors: (1) selenocysteine residue, (2) oligomerization status, and (3) GSH interacting residues of selenocysteine-containing GPx protein (**Figure 2**) [16, 18]. On the contrary to selenocysteine-containing GPx, the non-selenocysteine-containing GPx shown relatively low peroxidase activities [19] and thus their function serve as putative GPx are debated. In this context, the mechanism of how GPx7 and GPx8, two of the non-selenocysteine-containing GPxs that do not have GSH binding domain [15], carry out their oxidative stress-releasing function would be of highly interest. Previous studies demonstrated that GPx7 employs interacting proteins as mediators (**Figure 1**) [6, 20-22]. When cells expose to oxidative stress, GPx7 acts as an intracellular sensor that detects redox level and transmits ROS signals to redox-sensitive, thiol-containing proteins to facilitate the regulation of multiple biologic processes such as protein folding and releasing of the non-targeting short interfering RNAs (siRNAs)-associated stress. In this review, we will discuss the biochemical and biological roles of GPx7 in regulating thio-containing proteins in maintaining physiological redox homeostasis.

Structural and biochemical characterization of GPx7

Mammalian GPx7 is a non-selenocysteine containing phospholipid hydroperoxide glutathione

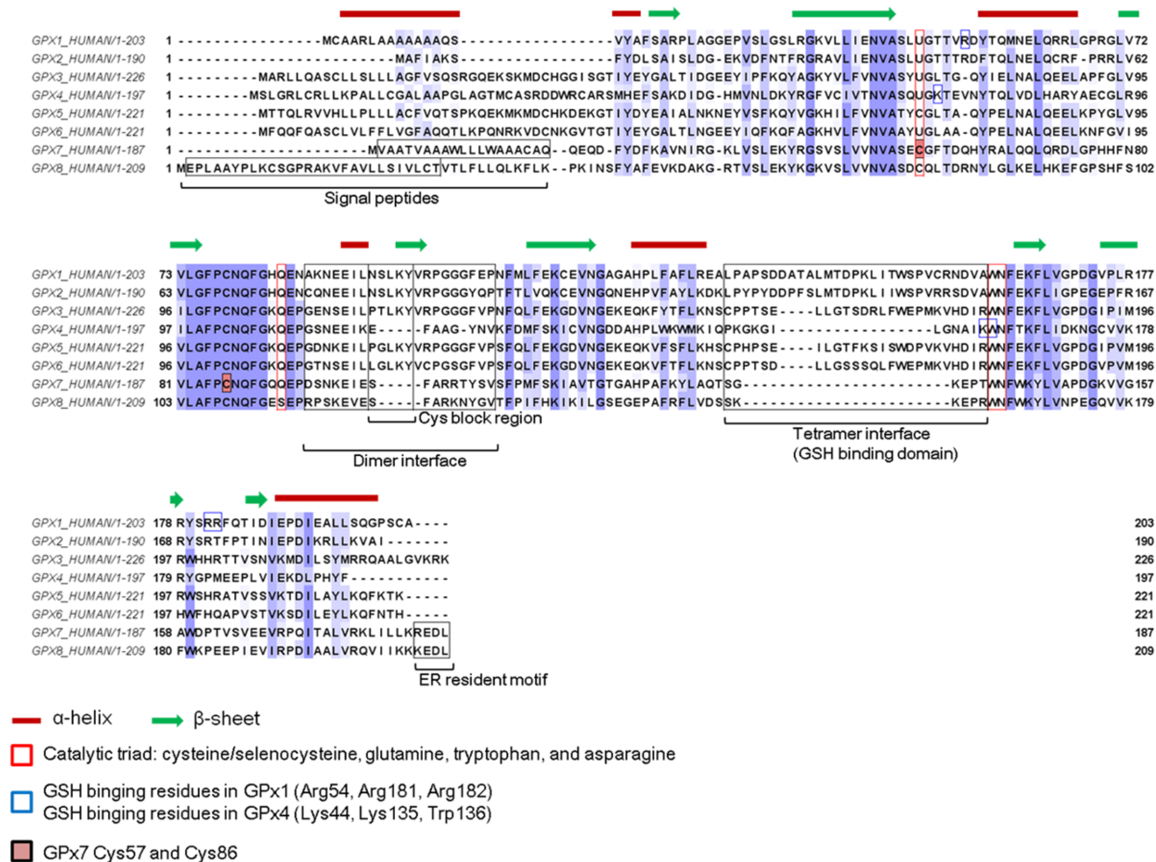

Figure 2. Alignment of amino acid sequence and domain structure of human GPxs. The similar amino acids among members of the family are shown in blue color. GPx5, GPx7 and GPx8 are non-selenocysteine containing GPx: cysteines (C) instead of selenocysteines (U) are present in the catalytic triad (red box). GPx7 and GPx8 have no GSH binding domain. GPx7 senses and transmits oxidative stress through disulfide shuttling (Cys57 and Cys86 marked by pink block). Since GPx7 has no resolving cysteine (C_R) in the cysteine block region, it is not a typical CysGPxs. GPx7 and GPx8 have ER retrieval motif for transportation and retention in the endoplasmic reticulum.

peroxidase, also known as NPGPx [14]. Mammalian *GPx7* gene contains three exons and encodes 187 amino acids with a predicted molecular mass of 22 kDa. GPx7 has two unique features which seen in ER secretory proteins: (1) an ER-signaling peptide at the N-terminus (1-19 a.a. of hGPx7 and 1-18 a.a. of mGPx7, respectively), and (2) an atypical KDEL motif at the C-terminus which serves as ER-retrieval signals [23]. Although the majority of GPx7 resides in ER, it also seems to present in other intracellular counterparts. Upon cleavage of the N-terminal signal, GPx7 could translocate from ER to the Golgi along the secretory pathway [24].

GPx7 does not contain the GSH-binding domain (**Figure 2**), which is involved in oligomerization and subsequent binding of GSH [15]. Ectopically expressed GPx7 from *E. coli* has minimal GPx activity [20]. X-ray crystallography-based structural analysis clearly distinguishes GPx7 from other GPx, as it is a non-selenocysteine containing monomeric ER secretory protein [16].

As a non-selenocysteine containing GPx, GPx7 encodes cysteine (Cys) at the catalytic sites (Cys57 and Cys86, **Figure 2**). Upon the ROS stimuli, the thiol moiety (-SH) of cysteine residues are oxidized and form either reversible disulfide bonds, including intramolecular disulfide bonds (R-S-S-R) and sulphenyl moiety (R-SOH), or irreversible sulphinic ($R\text{-}SO_2H$) and sulphonic ($R\text{-}SO_3H$) acid [22, 25-27]. Most of the GPxs in non-vertebrates including bacteria, fungi, insects and plants are cysteine-encoding GPx (CysGPxs). In CysGPxs, the N-terminal active cysteine oxidized by peroxide is called peroxidatic Cys (C_P). The resolving Cys (C_R) that locates in the cysteine block forms intramolec-

ular disulfide bridge with C_P, subsequently boosts its catalytic activity [16, 28]. The C_P-C_R disulfide bond is mainly reduced by the reducing thioredoxin, and the process subsequently turns the reduced thioredoxin into oxidized form. Thus, typical CysGPxs are thioredoxin peroxidases. On the other hand, GPx7 only equips with a peroxidatic Cys (C_P) and has no resolving Cys (C_R) within the cysteine block (**Figure 2**) that catalyzes the H_2O_2 reduction in the presence of thioredoxin [17, 22]. Cysteine residue Cys57 of GPx7 is the peroxidatic cysteine (C_P), which is the most vulnerable Cys in response to reactive oxygen species and is located in the -NVASxC(U)G-reactive (seleno) cysteine-containing motif. The other cysteine residue, Cys86, is not located in the cysteine block of typical CysGPxs where to form internal disulfide bond with C_P and interact with thioredoxin [17, 29]. Instead, it is located in the highly conserved -FPCNQF- motif found in all GPxs (**Figure 2**). Although Cys86 is not located in the typical site of C_R, it displays a unique feature via forming an intramolecular disulfide bond with Cys57. Taken together, the disulfide bond formation in GPx7 is not through thioredoxin peroxidase activity and differs from that of typical CysGPxs.

Affinity purified recombinant GPx7 protein exists in monomeric form and displays free thiol groups under reducing conditions. Treatment of reduced GPx7 (rGPx7) with H_2O_2 *in vitro* induces two monomeric forms of GPx: the intra-molecular disulfide bond containing GPx7, and the sulphinic/sulphonic cysteine-containing GPx7. Mass spectroscopy analysis of the former oxidized form of the GPx7 (oGPx7) revealed a single disulfide bond between Cys57 and Cys86. Based on the crystal structure of rGPx7 (PDB ID: 2p31), the distance between the two cysteine residues is 11.58 Å, which is longer than the average of other observed disulfide bonding cysteines. The distance of the predicted disulfide bond formation is typically between 3.0~6.2 Å, and a permanent disulfide bond is formed at a distance less than 3.0 Å [30]. Thus, oGPx7 might undergo a significant conformational change for the thiols to form an intramolecular disulfide. Such hypothesis is supported by the conformational changes of GPx7 upon H_2O_2 treatment based on tryptophan fluorescence wavelength shift showing Trp142 located close to the C_P active site [22]. Reversibly, the oxidized GPx7 can be reduced when treated with reducing agent such as dithiothreitol, ß-mercaptoethanol, or Tris(2-carboxyethyl)phosphine HCl (TCEP) [20]. In sum, these results indicate that oGPx7 contains a reversible intramolecular disulfide bond that might couple with the modification of GPx7-interacting redox-regulated proteins.

Identification of GPx7-interacting proteins and mechanisms of redox-regulated biochemical activities via disulfide bonding

GPx7 regulates multiple proteins to maintain redox homeostasis and to prevent ROS accumulation. Several proteins were found to interact with GPx7 covalently *in vivo* upon H_2O_2 stimulation based on an affinity pull-down assay with human osteosarcoma U2OS cells [20]. Among these interacting proteins, the most abundant proteins are peptide chaperones such as GRP78, GRP75, and ERp72 [20]. In response to oxidative stress, GPx7 facilitates the stress signaling transduction as well as stress releasing through interacting with its targeted proteins. Next, we will discuss the interacting mechanisms and functional consequences of the best-studied, GPx7 interacting proteins: GRP78, PDI, CPEB2, and XRN2.

GRP78

The 78-kDa glucose-regulated protein (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is a major chaperone of the endoplasmic reticulum (ER). ER is the biogenesis center of extracellular and organellar proteins. Under unfolded protein stress conditions, GRP78 binds to unfolded/misfolded proteins and subsequently releases and activates downstream ER stress transducers including IRE1, PERK, and ATF6 to trigger the unfolded protein response [31]. Unfolded protein response leads to translation attenuation, up-regulation of chaperones, and degradation of misfolded proteins [32]. To survive under ER stress, molecular chaperone GRP78 is induced and activated to facilitate the refolding process of misfolded protein [33].

The finding of significantly reduced protein refolding rate in GPx7-deficient cells revealed a crucial role of GPx7 on the protein folding process [20]. The mutant cystic fibrosis transmembrane conductance regulator (CFTR-Δ508) [34]

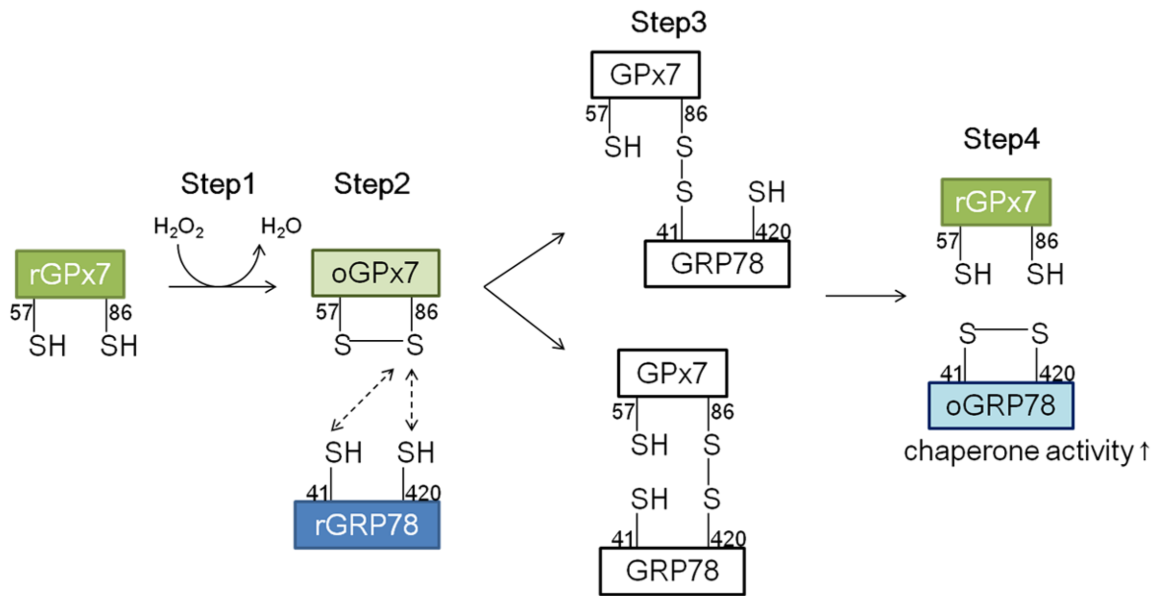

Figure 3. oGPx7 interacts with rGRP78 to facilitate GRP78 intramolecular disulfide bond formation and enhance its chaperone activity. The redox state of GPx7 is modulated by ROS through the modification of Cys57 and Cys86 (step 1). The switch of SH state in the cysteine residues changes the binding activity of GPx7. oGPx7 interacts with rGRP78 through Cys86 and transmits the disulfide bond to GRP78 (step 2 and 3). Finally, the chaperone activity of GRP78 is increased and the redox modification of GPx7 is reversed (step 4). Thus, GPx7 acts as a sensor protein for oxidizing GRP78.

and cytosolic protein chimera (GFP-250) [35, 36], refold poorly in the absence of GPx7. In response to the accumulation of misfolded proteins, a protective mechanism is activated to pack the misfolded proteins with vimentin, and it subsequently forms aggresome at the microtubule organizing center [37]. In GPx7-deficient mouse embryonic fibroblasts, ectopic expression of either CFTR-Δ508 or GFP-250 results in increases of aggresomes. In addition, binding of GRP78 to the misfolded CFTR-Δ508 and GFP-250 proteins was decreased in GPx7-deficient cells. A series of studies have provided mechanistic insights as how GPx7 and GRP78 facilitate protein-folding response [20].

Two cysteine residues (Cys41 and Cys420) of GRP78 are important for GRP78 chaperone activity. The binding ability of GRP78 to misfolded protein is reduced when both Cys residues are replaced by alanine residues ($GRP78^{C2A2}$). The interaction between GRP78 and GPx7 also requires both cysteine residues in GRP78. In contrast, only Cys86 of GPx7 is required for the interaction between GPx7 and GRP78. In the redox reaction, the role of Cys57 of GPx7 is different: Cys57 acts as an electron receptor to the Cys86-SOH triggering the GPx7 intermolecular disulfide bond formation. Such electron transfer activity is required for GPx7 function, which through its subsequent interaction with GRP78 results in the activation of GRP78 [20].

To elucidate the precise role of the Cys residues on GPx7 in GRP78 interaction, the interacting complex was analyzed by mass spectrum. Oxidized GPx7 (oGPx7) and reduced GRP78 (rGRP78) formed two forms of GPx7-GRP78 complexes *in vitro*: $GPx7^{Cys86}$-$GRP78^{Cys41}$ and $GPx7^{Cys86}$-$GRP78^{Cys420}$. Surprisingly, a novel form of GRP78 with Cys41-Cys420 intra-molecular disulfide bond was identified inadvertently in the oGPx7/rGRP78 mixture. Such kind of GRP78 was not detected in the H_2O_2-treated GRP78 without proper folding in the presence of GPx7, since the direct oxidation of GRP78 by H_2O_2 leads to sulfinic/sulfonic acid formation, which might result in an irreversible inactivation of GRP78. As summarized in **Figure 3**, GPx7 is oxidized by ROS and forms an intramolecular disulfide bond between Cys57 and Cys86 (step 1). The oGPx7 then interacts with rGRP78 (step 2) and forms GPx7-GRP78 intermediates (step 3). Subsequently, GRP78 forms the Cys41-Cys420 bond that facilitates its misfolded/denatured proteins-binding activity (step 4) [20].

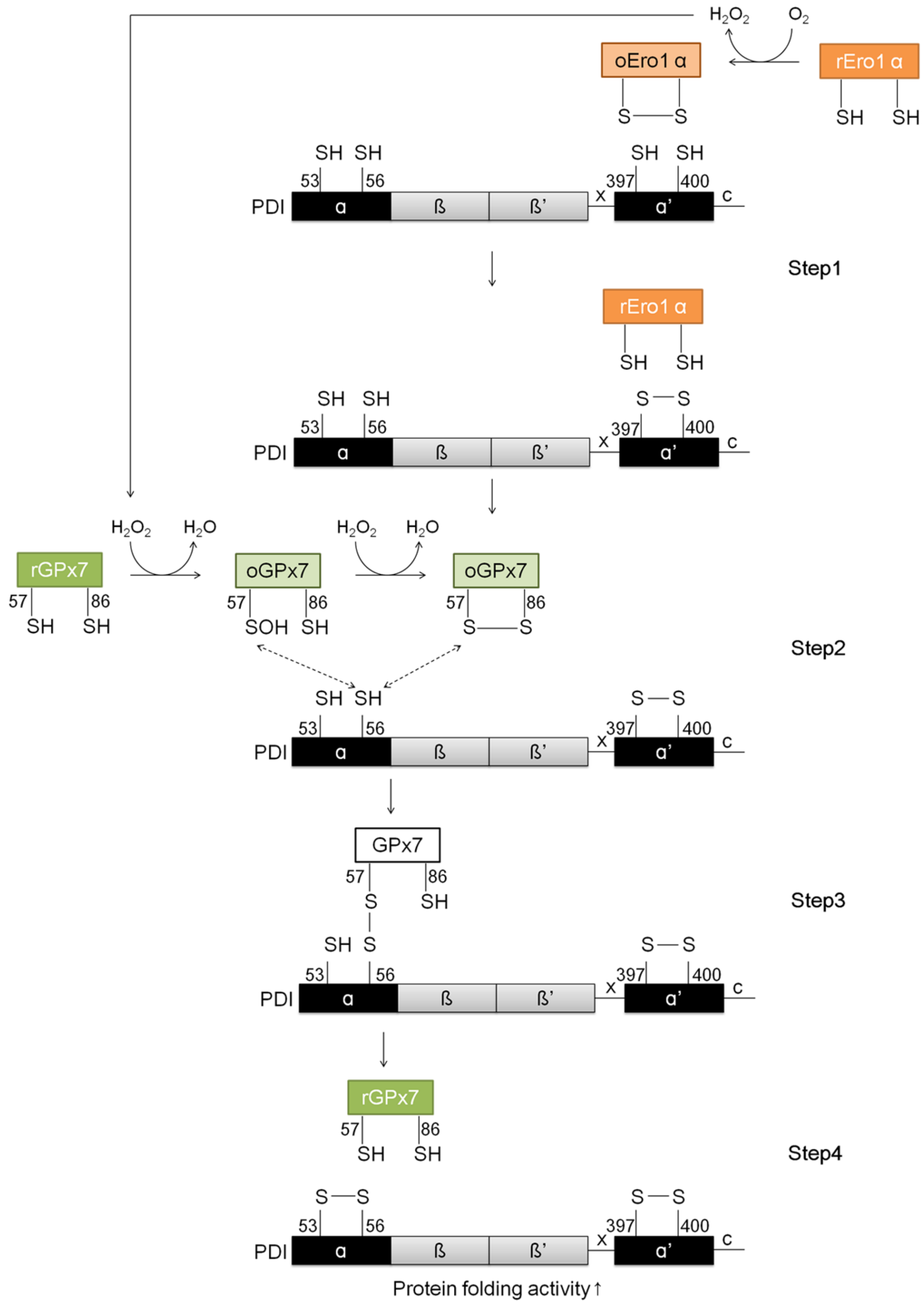

Figure 4. The activity of PDI to introduce disulfide bonds to ER-processed proteins is regulated by Ero1α and GPx7 in a sequential manner. Ero1α and GPx7 support PDI reactivity through ROS generating and consumption mechanisms, respectively. oEro1α and oGPx7 transfer the disulfide bonds to the α' and α domain of PDI to increase its protein folding activity.

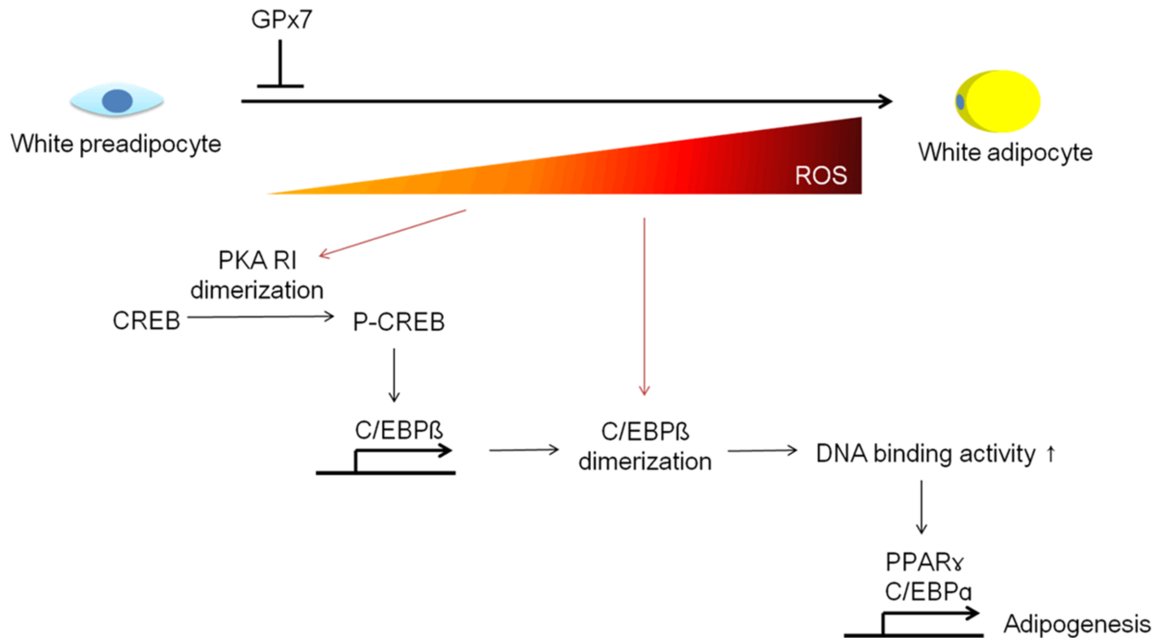

Figure 5. Role of GPx7 in adipocyte differentiation. Lack of GPx7 promotes adipogenesis through ROS dependent manners: (1) In the early phase of adipogenesis, increased dimerization of the PKA RI and phosphorylation of CREB leads to the induction of C/EBPβ [58]. (2) In the late stage of adipogenesis, increased S-S bond-induced dimerization of C/EBPβ acquires DNA binding activity to turn on two key adipogenic transcription factors: PPARγ and C/EBPα [87]. Abbreviations: C/EBP, CCAAT/enhancer-binding protein; CREB, cyclic AMP regulatory element-binding protein; P-CREB, phosphorylated CREB; PKA RI, protein kinase A regulatory subunit; PPARγ, peroxisome proliferator-activated receptor-γ.

PDI

The main source of ROS in the ER comes from intra-molecular disulfide bond formation during the maturation process of many secretary and membrane proteins. This process requires protein disulfide isomerase (PDI), ER oxidoreductin 1 (Ero1) and GRP78 chaperone [38]. Ero1-PDI axis is a major contributor to disulfide bond formation in the ER. PDI introduces (oxidoreductase) or rearranges (isomerase) disulfide bonds during protein folding [39]. It is composed of four thioredoxin domains (a-b-b'-a'). The N- and C-terminal domains (a and a') contain redox catalytic CXXC motifs and are involved in thiols oxidation, reduction, and isomerization for re-initiation of the disulfide bonds. The internal thioredoxin domains (b and b') are non-catalytic structural domains for substrate interactions [40]. Ero1 plays an essential role in sensing ER redox perturbation, and rapidly switch its activity to maintain a balanced oxidizing-reducing environment for proper protein folding process. In the oxidizing ER environment, activated Ero1 generates disulfide bond through O_2 consumption in the presence of its flavin cofactor, and then delivers the disulfide bond to proteins undergoing folding via PDI [41]. Notably, Nguyen *et al.* found that GPx7 can increase the H_2O_2 utility of PDI by measuring the NADPH consumption and GSSG formation. Furthermore, GPx7 promotes PDI-mediated oxidative protein folding machinery through disulfide bond rearrangement [15]. A recent study further provides mechanism on how GPx7 promotes PDI activities - as illustrated in **Figure 4**, that Cys57 of GPx7 is the key cysteine residue to form complex with Cys56 in the α domain of PDI. Ero1α and GPx7 bind to α' and α domain of PDI, respectively, in a sequential manner cooperating in the Ero1α/GPx7/PDI triad. In summary, GPx7 promotes the Ero1α-PDI protein folding machinery by recycling Ero1α-produced H_2O_2 to oxidize itself, transfer the disulfide bond to PDI that results in the up-regulation of PDI activity (**Figure 4**) [22].

CPEB2

CPEB2 is the RNA-binding protein reported to suppress HIF-1α RNA translation under normoxic condition [42]. Upon oxidative stress, CPEB2 dissociate from the HIF-1α RNA and consequently up-regulates translation of HIF-

1α RNA [42]. This process is regulated by GPx7 [43]. Under normoxic condition, the covalent interaction of GPx7 and CPEB2 promotes conformational rearrangement of CPEB2 to achieve optimal binding activity to HIF-1α RNA. Upon oxidative stress, GPx7-CPEB2 covalent interaction is disrupted and hence abolishes the binding of CPEB2 to HIF-1α RNA leading to HIF-1α over-expression. Thus, GPx7 deficiency enhances HIF-1α RNA translation under normoxia, while the stress induced HIF-1α synthesis is not detected following oxidative stress due to the lack of CPEB2-controlled HIF-1α translation [43].

GPx7 modulates CPEB2-controlled HIF-1α translation according to cellular redox status via thiol based redox regulatory process. Interestingly, although Cys157 of CPEB2 is the most critical residue to form disulfide bond with Cys57 of GPx7, substitution of all three cysteine residues (Cys157, 350, and 444) in the N-terminus of CPEB2 is required to completely abolish its binding to GPx7. Thus, the disulfide bonds may be rearranged dynamically upon GPx7-CPEB2 interaction and finally increase CPEB2 binding activity to HIF-1α RNA [43]. It would be of great interest to further explore how GPx7 regulates the sequential intramolecular disulfide bonding of CPEB2 between N-terminal cysteine residues and C-terminal RNA binding domain.

XRN2

The regulation of XRN2 by GPx7 is a serendipity finding during the siRNA knockdown experiment [21]. It has been shown that upon expressing the non-target siRNA (NT-siRNA) in cells, GPx7 expression was induced through a nucleolin-dependent transcriptional regulating process [44]. In GPx7-deficient cells, expressing excessive amount of NT-siRNA leads to cell growth retardation and apoptosis [21]. These findings suggest a role of GPx7 in resolving NT-siRNA-induced stress. However, GPx7 does not possess RNase activity that is required for the removal of the accumulated NT-siRNA, rendered its function a puzzle. Further studies suggested that GPx7 binds to an exoribonuclease, XRN2, which is participating in micro-RNA turnover [45]. The NT-siRNA stress releasing activity of XRN2 was abolished in GPx7-deficient cells, while re-expressing wild-type GPx7 *in vitro* restored the deficiency. The two cysteines of GPx7 are required for the covalent bonding between GPx7 and XRN2 and essential for XRN2 function [21, 44]; however, the biochemical mechanism of how GPx7 activates XRN2 is not known and it requires further investigation.

Loss of GPx7 induces systemic diseases

Deficiency of GPx7 does not lead to embryonic lethality in mice [14]. It is noted that excessive ROS leads to the accumulation of oxidative modified forms of proteins with irreversibly functional alterations and gives rise to diseases [46]. Consistently, the GPx7 knockout mice carry multiple abnormalities, including glomerulonephritis, splenomegaly, cardiomegaly, vasculitis, and fatty liver [20], which associate with the observations such as obesity, autoimmunity, tumors and shortened life span. The potential mechanisms of how deficiency of GPx7 causes these diseases are reviewed as following:

GPx7 and obesity

Epidemiological studies have shown a significant association between oxidative stress and excessive fat accumulation in human [47-50]. Oxidative stress is considered as the result of fat accumulation with subsequent inflammatory response [51, 52]. However, recent studies suggests that instead of being a consequence, oxidative stress may be a prerequisite for adipogenesis. ROS level increases during adipogenesis [6, 53]. The GPx-7 knockout mice fed with high fat diet (HFD) increase the ROS level *in vivo*, and lead to significant weight gain. These mice display adipocyte hypertrophy and hyperplasia, while no distinguishable amount of food intake between the knockout and wild-type mice is noted. Also, reduced energy expenditure is observed in the knockout mice during the dark/active phase compared to wild-type mice on HFD, whereas no difference is observed on chow diet. Spontaneous ambulatory activity is also decreased in the knockout mice on HFD but not on chow diet. The weight increment and activity reduction does not result from mitochondrial dysfunction in 4-month-old mice, since young GPx7 knockout mice show normal exercise capability, beta-oxidation, and mitochondrial oxidative phosphorylation. Furthermore, rectal temperature and expression of genes involved in thermogenesis in brown adipose tissue were not altered in the knockout

mice. These results suggest the weight gain on GPx7 mice is due to the increases of adipocyte size and number [6].

It is reported that ROS is a crucial factor in promoting adipocyte differentiation and insulin signaling [54-56]. GPx7 is highly expressed in pre-adipocytes but not in mature adipocytes, implicating that loss of GPx7 in pre-adipocytes may be prone to adipogenesis [6]. Indeed, when subjecting GPx7-knockdown 3T3-L1 pre-adipocytes or GPx7-deficient primary stromovascular fraction (SVF) for adipogenesis assay, these cells are more susceptible to insulin signaling and undergo robust adipogenesis. Such robust adipogenesis can be reverted by supplementing antioxidants, indicating as essential role of ROS in these cells. Consistent with this observation, loss of GPx7 causes the increase of the intracellular ROS concentration and sensitizes cells to excessive environmental oxygen [6]. Interestingly, the expression of C/EBPβ, the key adipogenesis transcription factor, is increased during the early phase of adipogenesis in GPx7-deficient preadipocytes. C/EBPß expression is primarily regulated by PKA/CREB, the upstream kinases in insulin signaling cascade. PKA is a heterotetramer composed by two regulatory domains and two catalytic domains. Adipogenic hormone stimulation promotes cAMP binding to the PKA regulatory subunit (PKA RI), leading to the breakdown of the PKA complex and the release of PKA catalytic subunit (PKA C-α) which phosphorylate CREB [57]. CREB in turn binds to the promoter of C/EBPß. In GPx7-deficient cells, dimerization of PKA RI [58] with the release of the PKA C-α, and the phosphorylation of CREB were increased, which support the induction of C/EBPβ in GPx7-deficient cells. At the late stage of adipogenesis, C/EBPβ further transactivates the downstream transcription factors C/EBPα and PPARγ to facilitate the full differentiation of adipocytes [59]. As the consequence of C/EBPβ induction in GPx7-deficient cells, the mRNA levels of C/EBPα and PPARγ increase during the later phase of adipogenesis. C/EBPβ is activated by ROS and forms dimer through intermolecular disulfide bonds, which has higher DNA binding activity. In GPx7-deficient cells, the ratio of C/EBPβ dimer to monomer was increased, facilitating the activation of downstream adipogenic cascade such as C/EBPα and PPARγ transactivation. The summary of this cascade is illustrated in **Figure 5**. Because GPx7 has not been demonstrated that it physically interact with proteins in insulin signaling, the molecular mechanisms of how imbalance of redox homeostasis in adipocytes leads to adipogenesis in GPx7-deficient cells remain to be addressed.

Single nucleotide polymorphism analysis (SNPs) using three independent cohorts (British, North Finland, and Han Chinese populations) revealed the genetic variant rs835337, which locates upstream of GPx7, is significantly associated with the BMI. Two other SNPs in strong linkage disequilibrium with rs835337, including rs7529595 and rs6588432, the SNPs downstream of the GPx7 gene, are also associated with BMI. Importantly, the rs835337 SNP has been shown to associate with lower GPx7 expression in abdominal fat depots, higher plasma malondialdehyde levels, and increases oxidative stress marker in human. Thus, GPx7 protects against fat accumulation and adipogenesis in mice and human via modulating cellular ROS level, which highlights the importance of targeting redox homeostasis in obesity management [6].

GPx7 and autoimmunity

The perturbation of UPR and redox homeostasis is closely related to autoimmunity [60, 61]. There are several plausible mechanisms: (I) Increased autoantigen formation: unrecoverable levels of ER stress leads to cell apoptosis releasing dead-cell components, which may provide sources of autoantigens [60]. In addition, GRP78 is overexpressed in rheumatoid arthritis patients and serves as autoantigen to elicit autoreactive B- and T-cell responses [62-64]. (II) Immunogenicity caused by aberrant protein folding: misfolded proteins such as HLA-B27 can be expressed on the cell surface and induce autoimmune disease ankylosing spondylitis [65, 66]. (III) Redox imbalance-induced immune cell hyperactivation: ROS regulate activities of immune cells, such as bactericidal activity of phagocytes and B cell receptor or T cell receptor signaling processes [61, 67]. Excessive ROS in immune cells provokes inflammation and breakdown of immune tolerance, resulting in the autoimmune pathogenesis [68].

GPx7-deficient mice developed several immune disorders, such as splenomegaly and glomerulonephritis with antibody deposition in kidney.

These abnormal renal phenotypes and autoantibody production in GPx7-deficient mice are similar with pathological symptoms of human lupus nephritis [20], implicating the potential functions of GPx7 in immune homeostasis. However, whether GPx7 indeed participates in autoimmune development, and how such a stress sensor maintaining cellular redox status affects which pathway, remains to be fully explored.

GPx7 and cancer

Direct involvement of GPx7 in cancer remains unclear though an increase of tumor incidence in GPx-7 knockout mice was observed [20]. GPx7-deficient mice developed sporadic cancers, including lung adenocarcinoma (16.7%), leukemia (6.3%), breast carcinoma (5%), sarcoma (6.3%), and lymphoma (T-, B-, or mixed-cell. 61.1%). Functional studies of GPx7 in cancer cells implicate a protective role against oxidative stress. Knockdown GPx7 by siRNA in breast cancer cells sensitizes the cells to polyunsaturated fatty acids-induced cell death, while ectopic expression of GPx7 eliminates this detrimental effect [14]. Similarly, esophageal adenocarcinoma cells with GPx7 expression have significantly increased viability and reduced apoptosis upon H_2O_2 treatment. On the other hand, GPx7 deficiency may have a role in tumorigenesis through suppressing NF-κB activation through ROS independent manner [69]. NF-κB, a key regulator of many genes involved in inflammation and immunity [70], is activated by multiple environmental stimuli such as stress, cytokines and oxidants. Loss of GPx7 in esophageal adenocarcinoma cells increases the phosphorylation levels of IKK-α/β and IκB-α, which are upstream kinases essential for NF-κB activation. Constitutively activated NF-κB signaling plays important roles during cancer progression [71]. However, how GPx7 suppresses NF-κB signal at which key point remains to be explored.

GPx7 and shortened lifespan

Up to date, numerous studies reveal that oxidative damage is increasing during the aging process in many organisms, and the accumulation of oxidative damage contributes to malfunction of cellular processes [72, 73]. Age-associated increase of oxidative damages in key ER chaperones (including GRP78, calnexin, and PDI) compromises their function [74, 75]. The median lifespan of GPx7 knockout mice decreased to 400 days from normal 760 days. Pathological examinations of moribund GPx7-deficient mice reveal increased incidence of cardiovascular disease and tumors, which are common age-associated diseases [20]. Since GPx7 deficiency leads to systemic oxidative stress and defects in ER stress sensing and UPR function, these reasons may contribute to multiple aging phenotypes in GPx7 knockout mice.

Future directions

Regulation of GPx7 and its targeting proteins

GPx7 interacts with numerous proteins including ADF, GRP75, HSP7C, ERp72, eEF1A-1, U-Tmod, ErJ3, and Histone H1b, in addition to the aforementioned GRP78, PDI, and XRN2 [20]. The biological significance of these interactions between GPx7 and binding proteins remains to be explored. For example, the ADF protein, which regulates cargo transportation together with Cofilin in the trans-Golgi network [76], U-Tmod (TMOD3), which binds to actin monomer to determine cell polarity [77], could be regulated by GPx7 via thiol-disulfide shuttling. On the other hand, GPx7 may cooperate with GRP75, which contains mitochondrial targeting sequences [78] and binds to transcription factors such as retinoic acid receptors [79] or p53 [80], to shuttle mitochondrial proteins into nucleus to regulate transcription [81]. Its currently unclear why non-ER resident proteins such as the elongation factor EF1A-1 (eEF1A-1), Histone H1b, or HSP7C interacts with GPx7 in response to oxidative stress. Elucidating the biological significance of these interactions may shed light on the multi-facet functions of GPx7 in maintaining redox homeostasis.

GPx7 and autoimmune diseases

GPx7 knockout mice developed several immune disorders, including glomerulonephritis and autoreactive antibody production [20], suggesting that GPx7 may have a role in immunity. An increase of apoptosis, high level of oxidative stress, and up-regulation of UPR genes were observed in their kidneys [20]. Since UPR involves at many levels with the innate and adaptive immune responses [60, 82, 83], abnormal UPR may provoke the bypass mechanisms of immune tolerance and contribute to

the development of autoimmune seen in GPx7-deficiency. Alternatively, GPx7 may directly regulate targeted proteins by its cysteine-dependent redox activity to maintain immune homeostasis. These detailed regulatory mechanisms are of great interest to explore.

Differences between GPx7 and GPx8

Evolutionary and structural analyses reveal that GPx7 and GPx8 are evolved from a branch of monomeric GPx4 (also known as phospholipid hydroperoxide GPx, PHGPx) and these two members are highly similar to each other [16]. Both are atypical GPxs as the result of lacking oligomerization interface for GSH binding and share very high similarity in amino acid sequences and domain structures (**Figure 2**). Both have functional peroxidatic and atypical resolving Cys residuals in comparable positions [17]. Similar to GPx7, GPx8 also has an atypical ER retrieval motif at the C-terminus, which is KEDL instead of the characteristic KDEL for ER luminal proteins or KKXX for ER transmembrane localization. However, unlike GPx7 which is secreted into ER luman, GPx8 is predicted as a type I transmembrane protein due to the hydrophobic domain at its N-terminus (**Figure 2**). Because of this hydrophobic region, GPx8's ER-signaling peptide is uncleavable and atypical, suggesting the N-terminal part is present upon GPx8 maturation. Combined both bioinformatic prediction and functional studies, it has been proposed that GPx8, a transmembrane protein with more restricted expression than GPx7, is an ER-resident protein [23].

It has been shown GPx8 can reduce oxidized PDI [15] and prevent Ero1α-derived H_2O_2 leaking by interacting with Ero1α, a member of the sulfhydryl oxidase Ero1 family, suggesting the functional domain of GPx8 is facing the ER luminal part as a type II transmembrane protein [84]. Based on bimolecular fluorescence complementation study, there are interactions between overexpressed Ero1α and GPx8 [15]. However, other approaches trying to detect the direct interaction between Ero1α or PDI and endogenous GPx8 *in vivo* are inconclusive. GPx8 is also involved in Hepatitis C viral (HCV) particle production identified by an unbiased screening of substrates of viral protease NS3-4A, suggesting the N-terminal cytosolic tip of GPx8 is removed during viral replication [85]. Although the catalytic activity of GPx8 is required to facilitate HCV particle production, the role and molecular mechanism of GPx8 and the significance of its cleavage in the HCV life cycle remain largely unknown. Structural and *in vitro* functional analysis revealed that GPx7 and GPx8 were highly similar to each other. However, they have distinct N-terminal regions, which change their membrane anchor and localization. This may implicate their diverse physiological roles. The precise biological function of GPx8 remains to be explored.

Conclusions

GPx7 is an important sensor for oxidative stress, ER stress, and NT-siRNA-induced stress. The oxidized and reduced form of GPx7 is determined by cellular redox status. Upon oxidative stress, GPx7 is activated and transmits the disulfide bonding to specific proteins and turn on their activities to eliminate the stress. For example, it facilitates protein folding in ER via regulating chaperones like GRP78 and PDI. On the other hand, NT-siRNA stress induces GPx7 protein expression mediated by nucleolin transactivation. Thus, the increasing activity and amount of GPx7 are both important for releasing stress. In addition, the indirect role of GPx7 in regulating signaling transduction has been characterized in the insulin and NF-κB signaling pathway through balancing redox homeostasis. Thus, unlike other GPx, GPx7 may uniquely have pleural roles in the maintenance of redox homeostasis.

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Disclosure of conflict of interest

None.

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