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2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications

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H_2O_2 is a reactive oxygen species that has drawn much interest because of its role as a second messenger in receptor-mediated signaling. Mammalian 2-Cys peroxiredoxins have been shown to eliminate efficiently the H_2O_2 generated in response to receptor stimulation. 2-Cys peroxiredoxins are members of a novel peroxidase family that catalyze the H_2O_2 reduction reaction in the presence of thioredoxin, thioredoxin reductase and NADPH. Several lines of evidence suggest that 2-Cys peroxiredoxins have dual roles as regulators of the H_2O_2 signal and as defenders of oxidative stress. In particular, 2-Cys peroxiredoxin appears to provide selective, specific and localized control of receptor-mediated signal transduction. Thus, the therapeutic potential of 2-Cys peroxiredoxins is clear for diseases, such as cancer and cardiovascular diseases, that involve reactive oxygen species.

A new paradigm in signal transduction: H_2O_2 signaling

Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) induce the production of intracellular H_2O_2, and this is required for subsequent protein tyrosine phosphorylation [1,2]. The discovery of these facts led to a change in the general concept of the role of H_2O_2, from a cytotoxic reactive oxygen species (ROS; Box 1) variant to a signaling messenger. Since then, several studies have shown that the intracellular H_2O_2 can be generated via the stimulation of a variety of growth factors, cytokines and G-protein-coupled receptors [3,4]. Thus, it is now generally accepted that membrane receptor-dependent signaling is accompanied by transient and intracellular H_2O_2 production. Yet there is no quantitative measurement method that gives an accurate concentration of intracellular H_2O_2. It is therefore difficult to resolve whether the cell is being subjected to H_2O_2-dependent signaling or to oxidative stress (Box 1).

Theoretically, H_2O_2 signaling and oxidative stress can be differentiated according to several criteria: (i) the production source; (ii) the elimination system; (iii) the existence of a specific target; and (iv) the intracellular distribution. For instance, oxidative stress can take the form of an intracellular oxidative burst that damages susceptible molecules diffusely and nonspecifically, whereas the H_2O_2 signal can be generated and eliminated by specific enzyme systems, and can also modify a specific target signaling molecule within a localized compartment.

Mammals have a diverse set of peroxidases that collectively function to reduce H_2O_2 to water. There are three classes of peroxidases: catalase, glutathione peroxidases (GPxs) and peroxiredoxins (Prxs). Catalase is a heme-containing H_2O_2 dismutase, exclusively localized in the peroxisome, a subcellular organelle devoted to oxidizing organic substances, and catalase is believed either to remove H_2O_2 that passively diffuses into the organelle or to prevent H_2O_2 leakage [5]. Five GPx isoforms are distinguishable by their tissue and subcellular distribution and substrate specificity [6–8]. Among them, GPx1, also called cGPx, is the only cytosolic enzyme that reduces H_2O_2 by utilizing the electrons donated by NADPH via the glutathione–glutathione reductase system. GPx1 is a selenoprotein that is produced at low levels and is dispensable in unstressed animals [6]. 2-Cys Prxs represent a new type of peroxidase, because they are coupled with thioredoxin and thioredoxin reductase, forming an electron-conveying system [9]. Thioredoxin reductase transfers electrons from NADPH to thioredoxin, which in turn reduces a disulfide linkage formed during the peroxidase reaction in 2-Cys Prxs. Thus far, five 2-Cys Prx isoforms have been recognized in mammals. On the basis of the peptide regions that harbor a conserved cysteine residue, they can be classified into typical and atypical 2-Cys Prxs (Figure 1) [10].

Distinct from catalase and glutathione peroxidase, the 2-Cys Prxs have several interesting molecular features. Firstly, their peroxidase activity relies exclusively on a cysteine residue in the N-terminal region. Secondly, they are fairly abundant proteins, present in an approximate range of 100 nanograms to a few micrograms per milligram of cellular protein [11]. Thirdly, they are distributed broadly in intracellular compartments: they are present in the cytosol, mitochondria, peroxisome, endoplasmic reticulum and, more importantly in the plasma membrane [10]. Fourthly, members of the 2-Cys Prx subgroup have a high degree of affinity for H_2O_2; that is, the H_2O_2 concentration to achieve half maximal activity is $<20\ \mu M$ in the presence of thioredoxin and thioredoxin reductase [11]. It is believed that the receptor stimulation triggers the generation of H_2O_2

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Box 1. Reactive oxygen species

Reactive oxygen species (ROS) are oxygen-derived reactive molecules. They include the superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH·), singlet oxygen (O·) and ozone (O_3) in nature; in biological systems, the former three species are significant. The superoxide anion is not freely diffusible through the lipid membrane because of its negative charge, but probably can penetrate cells via anion channels. The superoxide anion attacks iron–sulfur centers in respiratory chains and iron-responsive proteins. NADPH oxidases actively produce superoxide at the expense of NADPH in mammalian cells. H_2O_2 is both membrane permeable and relatively stable and oxidizes proteins, particularly methionine to methione sulfoxide or on highly reactive cysteine residues to cysteine sulfenic, sulfinic and sulfonic acids in sequence. Among the ROS, the hydroxyl radical is known to be the most reactive and toxic, and it rapidly attacks proteins, lipids and nucleic acids via a free radical chain reaction.

intracellularly from the plasma membrane, so that H_2O_2 meets its target signaling molecules immediately. Indeed, non-phagocytic isoforms of NADPH oxidase that are integral membrane proteins with six transmembrane α helices are implicated in PDGF-, angiotensin II- and insulin-dependent signaling [12]. For example, the PDGF receptor signals to NADPH oxidase via phosphatidylinositol 3-kinase and Rac [13,14]. The direct interaction of Toll-like receptor 4 with the NADPH oxidase 4 isoform has been reported [15]. It is also believed that intracellular H_2O_2 generated in response to receptor stimulation is in the sub-lethal range, that is, several micromolar or less. In this context, the molecular features of 2-Cys Prxs implicate them as prime candidates for H_2O_2 signal regulators, although their catalytic efficiencies are less than those of catalase and GPxs [10].

Here, we discuss recent developments regarding the functional roles of 2-Cys Prxs in receptor signaling and oxidative stress. These advances have provided new insights into the mechanism of H_2O_2-mediated signaling, and they suggest new potential therapeutic approaches in a variety of reactive ROS-related human diseases.

The function of 2-Cys Prxs in receptor signaling

Initial attempts to examine the involvement of 2-Cys Prxs in receptor signaling investigated whether 2-Cys Prxs can eliminate intracellular H_2O_2 generated as a result of receptor stimulation. It was shown that overexpression of Prx I and Prx II, the two cytosolic 2-Cys Prxs, suppresses PDGF- and EGF-dependent H_2O_2 production as well as tumor necrosis factor (TNF)-α-induced nuclear factor (NF)-κB transcriptional activity [16]. Similar studies have since followed using other ligands, such as thyrotropin (TSH), TNF-α, and TNF-related apoptosis-inducing ligand (TRAIL), as well as further work on EGF-dependent H_2O_2 production, in various cell types [17–20]. Collectively, these studies show that 2-Cys Prxs not only eliminate the levels of intracellular H_2O_2 that were increased upon receptor stimulation, but also suppress the downstream signaling responses, including NF-κB transcriptional activity, c-Jun N-terminal kinase (JNK) activity and apoptosis. None of these investigations, however, has provided a direct mechanistic analysis of how 2-Cys Prx influences the signaling event.

Recently, systematic studies on the signaling role of 2-Cys Prxs have been published. One of these found the differential regulation of different MAP kinases by Prx II [21]. Under conditions of TNF-α stimulation in which Prx II activity was either partially blocked using a dominant negative mutant version or was completely abolished by gene knockout, JNK and p38 MAP kinase activation was enhanced whereas the activation of extracellular signal-related kinase (ERK) was suppressed. Given the fact that all three MAP kinases are activated by exogenously added H_2O_2 [22], this is a surprising result in that, although it is structurally simple and reactive, endogenously produced H_2O_2 has a differential selectivity for different target signaling pathways. Another study demonstrated the regulation of PDGF signaling by Prx II [23]. It was asserted that the endogenous H_2O_2 regulated by Prx II targets the phosphorylation of the tyrosines at sites 579/581 and 857 in the PDGF receptor, but has no effect on any of the other tyrosine sites that are known to be phosphorylated (Figure 2), whereas exogenously added H_2O_2 was shown to induce the phosphorylation of all possible tyrosine sites. It was also shown that the site-selective response is controlled solely by Prx II and not by other peroxidases, and is achieved through the inactivation of the membrane-associated protein tyrosine phosphatases (PTPs). Although these studies delineate the selective and specific roles of H_2O_2 as a second messenger and Prx II as a signal regulator in PDGF signaling, they are unsuccessful in elucidating the exact molecular identity of H_2O_2 targets. Further investigations

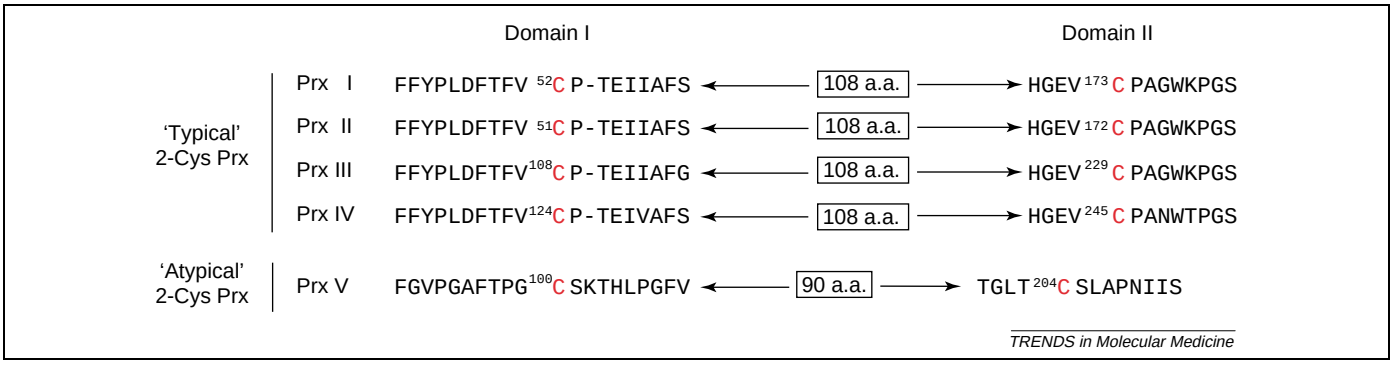

Figure 1. Sequence alignment of mammalian 2-Cys peroxiredoxins on the basis of peptide sequences around the cysteine residues that are involved in the peroxidase activity. The cysteine residues are colored red and their positions are numbered. The gap between Domain I and II is expressed as the number of amino acids (a.a.; boxed).

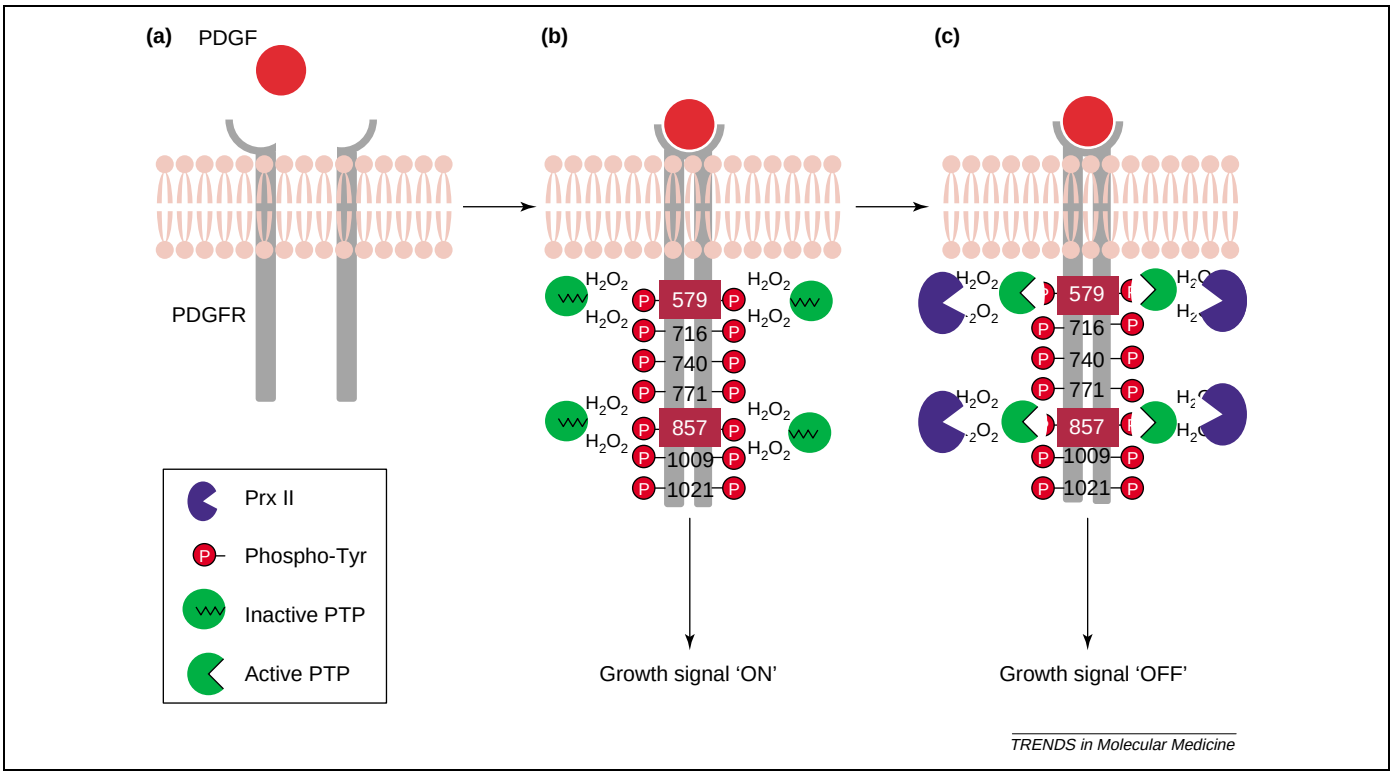

Figure 2. Hypothetical model for the cooperative action of Prx II, H_2O_2 and PTP in phosphorylation-site-selective regulation of PDGF receptor (PDGFR) signaling. Upon the engagement of PDGF **(a)**, the PDGFRs form homodimers and phosphorylate one another **(b)**. At the same time, H_2O_2 is thought to be produced transiently near the receptor. In this situation, a PTP in charge of dephosphorylation at tyrosine 579/581 and 857 is presumably located close to the receptor and is ready to react with phosphotyrosines at both sites. The generated H_2O_2 immediately oxidizes, however, and then inactivates the PTP. Phosphorylation at both sites is preserved, thereby activating the receptor tyrosine kinase (RTK). The RTK actively propagates the phosphorylation signal to downstream molecules, which eventually induces cell proliferation. Because of the necessity for signal attenuation **(c)**, Prx II is recruited by the activated receptor complex, eliminating H_2O_2. PTP is therefore activated and removes the phosphoryl groups from sites 579/581 and 857. RTK activity is consequently reduced, and the growth signal is diminished. In this model, the exact location of H_2O_2 generation and the presence of PTP responsible for dephosphorylating tyrosine 579/581 and 857 are tentative.

are needed to determine a molecular link between endogenous H_2O_2 and its downstream signaling.

Functional switching of 2-Cys Prxs under oxidative stress

In general, GPx and catalase activities are measured in the presence of 5 mM and 30 mM H_2O_2, respectively, to achieve their maximal activity [24,25]. By contrast, 2-Cys Prxs are irreversibly inactivated during the *in vitro* enzyme reaction in the presence of millimolar concentration of H_2O_2 [11]. Whether or not this unusual biochemical property is meaningful in a cellular context has recently been addressed intensively by several laboratories. An acidic shift of the 2-Cys Prxs has been shown with the use of 2D gel analysis of H_2O_2-treated cell lines [26–29]. Mass spectrometric analysis of the shifted acidic spot indicated that the reduced active site cysteine in one 2-Cys Prx was overoxidized to cysteine sulfinic acid ($-SO_2$). More importantly, this overoxidation was the cause of irreversible inactivation and was shown to require the complete peroxidase reaction cycle, such that 0.017% of active enzyme was overoxidized per reaction cycle [29]. The overoxidation is observed in cells challenged with oxidative stress or with long treatment with TNF-α [27,29], but not in growth factor-treated cells [23].

Overoxidation of 2-Cys Prxs was initially thought to cause a permanent loss of peroxidase function [11], but this has been shown to be incorrect by a series of recent publications. It has been shown in a cellular system that the 2-Cys Prx overoxidation is reversible [30]. Moreover, two novel enzyme systems – sulfiredoxin and sestrin, which induce a reversion of the cysteine sulfinic acid to reduced sulfhydryl in 2-Cys Prx – have been discovered and characterized in yeasts and mammals [31,32]. Sulfiredoxin, which is the better characterized of the two, uses ATP to reduce the cysteine sulfinic acid.

It has been claimed that 2-Cys Prxs undergo a functional change, from peroxidase to molecular chaperone, upon overoxidation in yeast and mammals [33,34]: the overoxidation results in a structural change from the dimer to a high-molecular-weight multimer that has molecular chaperone activity. Although the chaperone activity of the overoxidized 2-Cys Prx in cells has yet to be proven, it seems to be crucial for the survival of oxidatively damaged cells. The reversal of the overoxidation is kinetically slow (the turnover number is $3\times10^{-3}\,s^{-1}$) [35]; this makes sense, in that the chaperone function, if it exists, could this work throughout cell recovery.

The recently understood molecular features of 2-Cys Prxs enable us to postulate a schematic model for the cellular function of 2-Cys Prxs, in which they switch between H_2O_2-mediated stress and intracellular H_2O_2 signaling (Figure 3). In normally growing cells, 2-Cys Prxs catalyze the peroxidase reaction through which the H_2O_2 signal induced by receptor stimulation is attenuated. When cells are damaged to an abnormal degree by

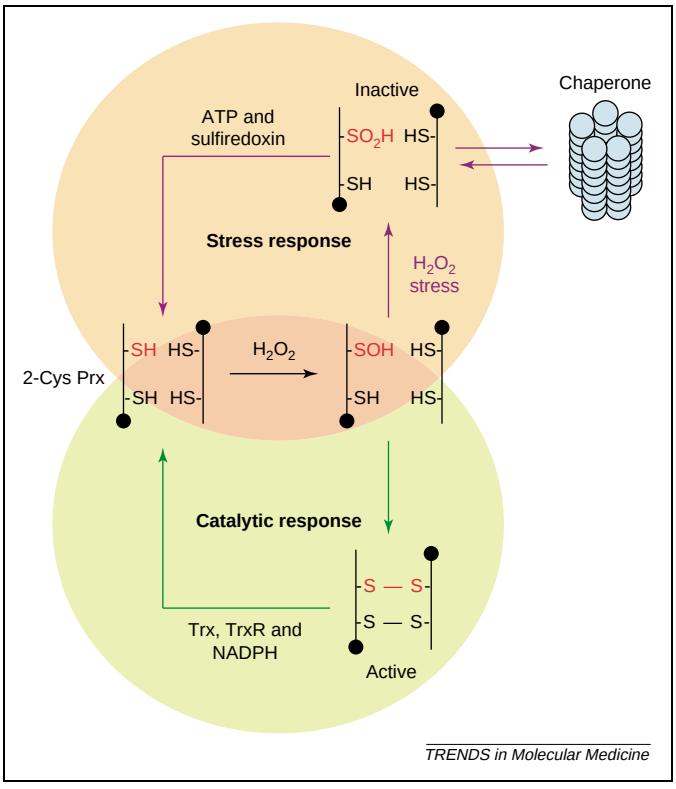

Figure 3. The dual function of 2-Cys Prxs in cellular physiology. Catalytic response: In normally growing cells, in which H_2O_2 generation is transient or pulsed, 2-Cys Prx efficiently controls H_2O_2 concentration through a canonical reaction cycle coupling with thioredoxin (Trx) and thioredoxin reductase (TrxR). Stress response: Under oxidative stress, however, 2-Cys Prx initially undergoes a canonical reaction cycle but cannot avoid overoxidation to cysteine sulfinic acid (-SO_2H), as a result of the persistent peroxidase reaction. The overoxidized 2-Cys Prx proteins then multimerize into a high molecular weight chaperone complex, which presumably protects the cells from protein aggregation. If the oxidative stress is weakened, the overoxidized 2-Cys Prx is gradually converted to active enzyme by sulfiredoxin in the presence of ATP. This model would be confirmed when it has been proved that 2-Cys Prx overoxidation does not occur during receptor-mediated signaling, which is a highly controversial issue.

exogenously added H_2O_2, however, 2-Cys Prxs become subjected to an overoxidation and multimerization, which in turn could endows them with a function as chaperones. Their role appears to be preventing protein aggregation and thereby assisting cellular recovery. If cells survive, the overoxidized 2-Cys Prxs could be re-reduced by sulfiredoxin and become available for normal receptor signaling.

It has been discussed, however, that mammalian 2-Cys Prxs are structurally sensitive to overoxidation [36] and 2-Cys Prx effects on H_2O_2-mediated cell signaling would probably occur only in a very restricted region localized around each receptor [23]. Thus, it might be that only a very small percentage, perhaps an undetectable amount, of the 2-Cys Prxs is overoxidized near the receptor, even if this is crucial in enabling the peroxide levels to rise and support a signal. By contrast, a 2-Cys Prx appears to be recruited to the receptor in PDGF stimulation [23], suggesting that 2-Cys Prxs stay in the cytosol in resting cells. This is a controversial issue that needs further investigation. Interestingly, the appearance of overoxidized 2-Cys Prxs has been regarded as an immediate marker to indicate that cells face the oxidative stress [37].

2-Cys Prxs in human diseases

Diseases that involve abnormal inflammatory and metabolic processes, such as cardiovascular dysfunction, cancer, diabetes mellitus and neurodegeneration, often also involve abnormal control of ROS. For example, some blood cells such as monocytes, macrophages and platelets, when recruited to atherogenic vessel walls, generate ROS, which then oxidize low-density lipoproteins (LDLs) and activate endothelial and smooth muscle cells [38,39]. The hyperproliferative property of cancer cells is known to be associated with increased production of intracellular ROS [40]. ROS might also induce aberrant protein aggregation in a variety of neurodegenerative disorders [41].

Although ROS are recognized to be important in these life-threatening diseases, whether they are involved in initiation of the diseases or are simply a consequence of the disease progression is still uncertain. An important clue will come when a physiological regulator of intracellular ROS is identified, which would help us to elucidate the mechanism and further facilitate target-oriented therapy for ROS-related diseases.

Many reports have claimed an association between alterations in the protein level of 2-Cys Prx isoforms and these human diseases; for example, all forms of 2-Cys Prxs are seen to be increased in lung and breast cancers [42–45] . In addition, Prx I expression is also shown to be increased in esophageal cell carcinoma, pancreatic adenocarcinoma, and oral/thyroid cancers [46–49]. Prx I expression is also elevated in breast cancer patients not responding to docetaxel [50]. By contrast, Prx II expression is decreased in urinary bladder cancer [51], whereas Prx III is overexpressed in hepatocellular carcinomas [52]. Prx IV is decreased in stomach cancers [53], which might be significant in that Prx IV is downregulated in Ras-transformed cells [54]. Because these data have been obtained through immunological or proteomic analysis of the diseased tissue, the cellular model of how, if at all, 2-Cys Prx is involved in tumorigenesis is not clear.

Prx I and Prx II are differentially expressed in brain regions [55]: Prx I is expressed in astrocytes, whereas Prx II is expressed in neurons. These data indicate that the two 2-Cys Prxs have distinct functional roles in the brain and provide differential contributions to neuropathologic conditions. The expression patterns of 2-Cys Prxs are in fact highly variable in different regions of the brain during neurodegenerative disease processes. Prx I is decreased in the frontal cortex of patients with sporadic Creutzfeldt–Jacob disease [56]. Prx II is increased, whereas Prx III is decreased, in Alzheimer's disease and Down Syndrome [57]. Prx III is involved in the protection of hippocampal neurons from excitotoxic injury [58]. Given that oxidative damage is involved in the pathogenesis of neurodegenerative diseases, the alteration in 2-Cys Prx expression would appear to be primarily a consequence of cellular resistance to the oxidative damage.

A limited number of studies have suggested an involvement of 2-Cys Prx in cardiovascular diseases and diabetes mellitus. One report claimed that transcription factor Nrf2, one of the erythroid transcription factor NF-E2 subunit factors responsible for the induction of defense proteins including Prx I [59], is activated in oxidized

LDL-treated macrophage cells, thus indirectly suggesting an involvement of Prx I in macrophage activation [60]. It has recently been shown that Prx II suppresses the proliferation and migration of smooth muscle cells (SMCs) through the site-selective phosphorylation of the PDGF receptor and, furthermore, is involved in the neointimal thickening of SMCs in balloon-injured carotid artery [23]. Prx I and Prx II are upregulated in pancreatic β cells when stimulated with cytokines, hydrogen peroxide, and streptozotocin suggesting an involvement in the pathogenesis of diabetes mellitus [61]. Finally, Prx II is readily detected in the blood of severe acute respiratory syndrome patients, whereas it is undetectable in normal control blood [62], suggesting a potential use for it as a diagnostic biomarker.

The therapeutic potential of 2-Cys Prxs

Although alterations in the expression of 2-Cys Prxs in diseased samples appear to be relevant, the current data are insufficient for the determination of pathogenic mechanisms. Nonetheless, several *in vitro* and *in vivo* studies have implicated the potential of 2-Cys Prxs as either therapeutic targets or diagnostic biomarkers for major diseases.

Prx I expression is elevated in most cancers, and is increased as the result of carcinogen phorbol ester treatment, which affects the protein kinase C and p38 MAP kinase pathways [63]. Prx I is also known to be involved in tumor suppression, however, functioning as a c-Abl tyrosine kinase inhibitor [64] and as an inhibitor of Myc-mediated signaling by interacting with the Myc Box II domain of Myc [65]. Prx I-deficient mice consistently develop malignant tumors when aged [66]. Hence, the elevation of Prx I expression in cancer tissues has been hypothesized to be a consequence of self defense against tumorigenesis.

As discussed above, Prx II negatively regulates growth factor signaling and, thus, also negatively regulates cell proliferation. Given that growth factor-induced tyrosine phosphorylation is dependent on the generation of intracellular H_2O_2 [1,2], it has been hypothesized that self-sufficiency in growth signals, one of the crucial factors for tumorigenesis [67], is accompanied by the ligand-independent amplification of H_2O_2 signal. In this context, Prx II has been surmised to be a fundamental intracellular regulator that determines the amplitude of intracellular H_2O_2 appropriate for hyperproliferation but insufficient for apoptosis. In support of this hypothesis, the suppression of Prx II expression using antisense cDNA has been demonstrated to render cancer cells more susceptible to radiation-induced apoptosis [68]. Prx III knockdown by RNA interference also appears to sensitize cervical cancer cells to death receptor- and stress-mediated apoptosis, possibly through the disruption of mitochondrial function [69]. Prx III has been reported to be a Myc target gene product [70]. Considering these data collectively, a plausible therapeutic strategy for the treatment of cancer might be to screen for chemical drugs that target Prx II or Prx III activity, rather than targeting their transcriptional control. This approach could prove useful when applied in conjunction with radiation therapy.

In addition, Prx I and Prx II were initially cloned from K562 human erythroleukemic cells as natural killer cell enhancing factor (NKEF) A and B, respectively [71]. Recently, it has been reported that the splenic NK cells from Prx I-deficient mice show the reduced cytotoxic activity and *Prx* $I^{-/-}$ red blood cells do not appear to enhance the activity of the NK cells [66]. Thus, the possibility that recombinant 2-Cys Prx proteins could be useful for cancer prevention cannot be excluded. Similarly, recombinant Prx I and Prx II exhibit anti-viral activity, inhibiting HIV-1 replication [72].

Cardiovascular dysfunction is a chronic inflammatory process that spreads from the fatty streak and foam cells through lesion progression and plaque formation to plaque rupture and thrombosis [73]. Its early diagnosis and prevention is therefore unfeasible. During the lesion progression stage of the atherogenic process, SMCs migrate from the medial layer to the intimal layer of the arterial wall, proliferate, and form fibrous plaques. In this regard, a recent result showing the specific involvement of Prx II in SMC proliferation [23] implies the potential therapeutic value of Prx II for inhibiting atherogenic lesion progression.

In the study of neurodegenerative diseases, it is the chaperone activity of 2-Cys Prx that warrants attention, mainly because protein aggregation is involved in these diseases [33]. The chaperone activity of 2-Cys Prx is a result of overoxidation, presumably occurring in parallel with the aggregation of other oxidatively damaged proteins. Perhaps in this case, as there is no method established for the *in situ* amplification of 2-Cys Prx chaperone activity, the transcriptional activation of 2-Cys Prx expression or alternatively the use of recombinant 2-Cys Prx protein would be good ways to prevent abnormal protein aggregation in the damaged neurons. It was therefore an interesting observation that systemically administered recombinant Prx V has a protective effect against excitotoxic stress induced by ibotenate, which acts on the N-methyl-D-aspartate receptor [74].

Need of 2-Cys Prx isotype-specific inhibitors

Although 2-Cys Prxs share the same catalytic mechanism, it is evident that their roles *in vivo* are distinct. For example, Prx I is phosphorylated (predominantly by Cdc2) in mitotic cells, whereas Prx II is not [75]. Also, PDGF signaling is regulated solely by Prx II. Prx I-deficient mice develop malignant tumors in various tissues, whereas Prx II-deficient mice tend to develop only red blood cell abnormalities. The development of isotype-specific inhibitors is therefore necessary.

The crystal structures of several mammalian 2-Cys Prxs, including Prx I, Prx II and Prx V, have been resolved. Although Prx I and Prx II undergo intermolecular disulfide formation upon oxidation, whereas Prx V undergoes intramolecular disulfide formation, their structures are very similar [10]. Briefly, the N-terminal active site cysteine residue is deeply buried, whereas the counterpart C-terminal cysteine residue is partially exposed to the solvent. In all three structures, the sulfur atoms of the

active site and the resolving cysteine residues are relatively far part (~13 Å), so the formation of intermolecular or intramolecular disulfide linkage needs a conformational change. The active site cysteine residue interacts with Arg127 (numbering is based on the Prx II amino acid sequence), which appears to contribute to the active site reactivity, that is, lowering the p*K*a of the active site cysteine, at a distance of 3.3 Å.

The regional structure that surrounds the active site pocket appear to differ between 2-Cys Prxs, however. The Cys52 in Prx I is surrounded by several hydrophobic residues within the chain [76], whereas access to the Cys51 in Prx II is restricted by the Phe81 in the adjacent dimer [77]. The active site of Prx V comprises a positively charged pocket, which remains largely exposed to the solvent exterior [78]. Therefore, it might be possible to design active site-directed chemical inhibitors specific to each 2-Cys Prx isoform.

As well as the active-site-targeted inhibition of the 2-Cys Prx activity, activity could be inhibited by surface binding. This idea is supported by the presence of additional hydrophobic patches in Prx I, which are proposed to interact with the heme group [76], and by the inhibition of Prx I activity by Cdc2-dependent phosphorylation [75]. In addition, 2-Cys Prx inhibitors are postulated to be more useful than inhibitors of thioredoxin and thioredoxin reductase for controlling H_2O_2 signaling, because the latter enzymes have diverse cellular functions (e.g. they also couple with ribonucleotide reductase).

Concluding remarks

The biological function of 2-Cys Prxs has been uncovered only recently. 2-Cys Prxs appear to provide differential, selective and specific regulation of receptor signaling, and also to regulate intracellular signaling via direct protein–protein interactions [64,65]. Upon oxidative stress, 2-Cys Prxs undergo overoxidation of the active site cysteine, which causes loss of the peroxidase function. Interestingly, 2-Cys Prxs appear to gain a new function as molecular chaperone by overoxidation, although cellular activity has yet to be confirmed. Together, these recent data have highlighted the diverse and important roles of 2-Cys Prxs in intracellular H_2O_2 signaling and oxidative stress.

To accelerate the translation of 2-Cys Prx function for clinical application, the following questions should be addressed on future research. Can 2-Cys Prx direct selective and specific regulation of H_2O_2 signaling in other receptor tyrosine kinase signaling pathways? In particular, it will be interesting to study 2-Cys Prx effects on insulin-receptor-, EGF-receptor- and vascular-endothelial growth-factor receptor-mediated signaling further, because these receptors are important in various human pathophysiological conditions. What is the *bona fide* target molecule of endogenous H_2O_2 in a particular signaling pathway? This will provide new insights into the mechanism by which 2-Cys Prxs regulate H_2O_2 signaling. And finally, what is the quantitative level of H_2O_2 that triggers the switch between signaling and stress? To answer this question, a novel probe for the quantitative measurement of intracellular H_2O_2 must be developed.

Because H_2O_2-mediated signaling is being implicated more and more in human diseases, its control via 2-Cys Prxs promises clinical benefits. The control of either the expression or the activity of 2-Cys Prxs might therefore lead to a new generation of therapies targeting H_2O_2 signaling components.

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