The phospholipase A$_2$ activity of peroxiredoxin 6

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Abstract Peroxiredoxin 6 (Prdx6) is a Ca$^{2+}$-independent intracellular phospholipase A$_2$ (called aiPLA$_2$) that is localized to cytosol, lysosomes, and lysosomal-related organelles. Activity is minimal at cytosolic pH but is increased significantly with enzyme phosphorylation, at acidic pH, and in the presence of oxidized phospholipid substrate; maximal activity with phosphorylated aiPLA$_2$ is \( \sim 2 \) µmol/min/mg protein. Prdx6 is a "moonlighting" protein that also expresses glutathione peroxidase and lysophosphatidylcholine acyl transferase activities. The catalytic site for aiPLA$_2$ activity is an S32-H26-D140 triad; S32-H26 is also the phospholipid binding site. Activity is inhibited by a serine “protease” inhibitor (diethyl p-nitrophenyl phosphate), an analog of the PLA$_2$ transition state \([\text{1-hexadecyl-3-}-(\text{trifluoroethyl})-\text{sn-glycerol-2-phosphomethanol (M}35)]\), and by two naturally occurring proteins (surfactant protein A and p67$^{\text{phox}}$), but not by bromoenol lactone \( \text{aiPLA}_2 \) activity has important physiological roles in the turnover (synthesis and degradation) of lung surfactant phospholipids, in the repair of peroxidized cell membranes, and in the activation of NADPH oxidase type 2 (NOX2). The enzyme has been implicated in acute lung injury, carcinogenesis, neurodegenerative diseases, diabetes, male infertility, and sundry other conditions, although its specific roles have not been well defined. Protein mutations and animal models are now available to further investigate the roles of Prdx6-aiPLA$_2$ activity in normal and pathological physiology.—Fisher, A. B. The phospholipase A$_2$ activity of peroxiredoxin 6. J. Lipid Res. 2018, 59: 1132–1147.

Peroxiredoxin 6 (Prdx6) is a member of the peroxiredoxin family of enzymes that function as cysteine-dependent peroxiredoxins with primary physiological roles in cell signaling and antioxidant defense (1). Prdx6 is similar to other peroxiredoxins in its peroxidase activity toward small peroxides such as H$_2$O$_2$, but it also exhibits several important characteristics that distinguish it from other members of the peroxiredoxin family (2). These characteristics of Prdx6 include: 1) a catalytic mechanism for peroxidase activity that depends on a single conserved Cys in contrast to the 2-Cys mechanisms for most other peroxiredoxins (3); 2) the utilization of GSH as its primary physiological reductant for peroxidase activity (4, 5), in contrast to the utilization of thioredoxin as the primary reductant for other peroxiredoxins (3); and 3) the ability to bind phospholipids (6, 7) with subsequent activities to: a) reduce phospholipid hydroperoxides to the corresponding alcohol, i.e., phospholipid hydroperoxide glutathione peroxidase activity (PHGPx) (8); b) hydrolyze the sn-2 fatty acyl bond of phospholipids, i.e., phospholipase A$_2$ activity (PLA$_2$) (4, 9); and c) transfer a fatty acyl COA into the sn-2 position of lysophosphatidylcholine (LPC), i.e., LPC acyl transferase (LPCAT) activity (10). The binding of phospholipids and the associated three lipid-related enzymatic activities of Prdx6 (PHGPx, PLA$_2$, and LPCAT) are not expressed by other members of the peroxiredoxin family. Thus, Prdx6 is a unique multifunctional protein.

Despite the major physiological and pathophysiological importance of the PLA$_2$ activity of Prdx6, it has received relatively limited attention in reviews devoted to PLA$_2$ enzymes (11–16) or in general reviews of peroxiredoxins (1, 3, 17, 18). While reviews focused specifically on Prdx6 have contrasted its peroxidase and PLA$_2$ activities, they have generally emphasized the former (2, 19–22). The present

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Abbreviations: AA, arachidonic acid; aiPLA$_2$, acidic Ca$^{2+}$-independent PLA$_2$; ALI, acute lung injury; BALF, broncho-alveolar lavage fluid; BEL, bromoelonal lactone; cPLA$_2$, cytosolic PLA$_2$; DENP, diethyl p-nitrophenyl phosphate; DPPC, dipalmitoyl phosphatidylcholine; GPx, GSH peroxidase; LB, lamellar body; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPCAT, LPC acyl transferase; LPLA$_2$, lysosomal PLA$_2$; LRO, lysosomal-related organelle; M35, 1-hexadecyl-3-((trifluoroethyl)-sn-glycerol-2-phosphomethanol; NOX1, NADPH oxidase type 1; NOX2, NADPH oxidase type 2; PAFAH, platelet-activating factor acetyl hydrolase; PC, phosphatidylcholine; PG, phosphatidylglycerol; PHGPx, phospholipid hydroperoxide GPx; PLA$_2$, phospholipase A$_2$; Prdx6, peroxiredoxin 6; ROS, reactive oxygen species; SDH, Ser-Asp-His; SP-A, surfactant protein A.

This review is dedicated to the memory of Dr. Mahendra Jain, deceased September 22, 2017; Mahendra was an inspirational colleague who helped to guide the initial studies of PLA2 enzymology in my laboratory, providing insights that otherwise were unavailable.

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This article will review the unique PL\(A_2\) activity of Prdx6 and its roles in normal and pathologic physiology.

**PRDX6 AS A PL\(A_2\)**

A moonlighting protein

The presence of a novel PL\(A_2\) activity with an important role for lung phospholipid metabolism and with characteristics subsequently associated with Prdx6 was discovered as a result of experiments that used isolated lungs and epithelial cells in culture (23, 24). The protein was identified and subsequently cloned through use of a novel inhibitor of PL\(A_2\) activity and assay at acidic pH (25). Although the originally published molecular sequence for Prdx6 was based on its PL\(A_2\) activity (25–27), it shortly thereafter became apparent that the protein had homology to the peroxiredoxin family, and, like other family members, it also expressed peroxidase activity (4, 28). Based on that finding, several subsequent articles reviewing PL\(A_2\) enzymes regarded Prdx6 as a peroxidase and, therefore, not a PL\(A_2\) (12, 13). However, it is now well recognized that many proteins can perform more than one function, and, although their trivial names may reflect only one activity (for example, peroxiredoxin implies a peroxidase), the “unnamed” second function may be of equal importance (29–31). This multifunctional activity has led to the appellation “moonlighting” protein. Dual functionality for a protein may include one enzymatic activity and a structural function, as shown by the crystallins, for example (32), but also might include two distinct enzymatic activities such as cytochrome c that functions in the TCA cycle as well as in the pathway to cellular apoptosis (33). While Prdx6 is called a peroxiredoxin, it has both PL\(A_2\) as well as GSH peroxidase (GPx) activities. Interestingly, Prdx6 also expresses a third enzymatic activity, LPCAT, that is coupled to its PL\(A_2\) activity (10), and perhaps a fourth (nongenetic) function, namely, a molecular chaperone activity as recently described for a Prdx6 homolog in cyanobacterium (34).

Evidence that Prdx6 is a PL\(A_2\)

There are several lines of evidence confirming the PL\(A_2\) activity of Prdx6. First, activity of recombinant Prdx6 is demonstrated by the liberation of a FAA from the sn-2 position of phospholipids (2, 4, 5, 27, 35–38). Although a prior review of PL\(A_2\) enzymes characterized the Prdx6-PL\(A_2\) activity as “trace” (13), measured rates of activity range from ~100 to 130 nmol/min/mg for unmodified Prdx6 to almost 2 \(\mu\)mol/min/mg protein for Prdx6 that has been phosphorylated (35, 36, 39). While this activity is considerably lower than the activities expressed by snake venoms and other secreted PL\(A_2\) enzymes, it is similar in magnitude to that shown by other intracellular PL\(A_2\) enzymes—for example, human platelet PL\(A_2\) (40), Ca\(^{2+}\)-independent PL\(A_2\) from a macrophage cell line (41), lysosomal PL\(A_2\) (LPL\(A_2\)) (42), and Photobacterium dancelae PL\(A_2\) (43).

The second line of evidence for Prdx6-PL\(A_2\) activity is the molecular sequence of Prdx6 that contains the motif GxSxG (amino acids 30–34) (25–27), a sequence that has been associated with lipase activity in other proteins (20, 21, 44). Furthermore, S32-H26-D140 has been identified as the PL\(A_2\) catalytic triad in Prdx6 (6); this triad is commonly expressed in proteases and proteins with PL\(A_2\) activity (20, 44–47). The critical S32 residue is conserved in Prdx6 from mammals (human, bovine, rat, and mouse), birds (chicken, Gallus gallus), amphibians (frog, Xenopus tropicalis), fish (Atlantic rock cod, Trematomus bernacchii), insects (Drosophila dPrx 2540 and dPrx 6005), plants (cress, Arabidopsis thaliana), and yeast (see GenBank\(^{TM}\) and refs. 25–27, 48, and 49). The Drosophila, plant, and yeast proteins are 1-cysPrdx enzymes that are homologous to Prdx6. Although the S32 site is conserved in the Prdx6 homologous proteins, PL\(A_2\) activity, so far, has been reported only for the mammalian enzyme.

A third line of evidence for Prdx6-PL\(A_2\) activity is the inhibition of activity by a designed analog of the PL\(A_2\) transition state (23, 50, 51). The transition state analog inhibitor, called 1-hexadecyl-3-(trifluoroethyl)-sn-glycerol-2-phosphomethanol (MJ33), is described below.

As a fourth line of evidence, PL\(A_2\) activity, measured under conditions that are relatively specific for Prdx6 (acidic pH, absence of Ca\(^{2+}\), and inhibition by MJ33), has been demonstrated in lung tissue and cellular homogenates. The PL\(A_2\) activity in freshly isolated lung homogenate was 0.15 nmol/min/mg protein and, in alveolar type 2 epithelial cells that were stimulated with a phorbol ester, was ~0.7 nmol/min/mg protein (24, 52), a value 7-fold greater than the activity of Ca\(^{2+}\)-independent PL\(A_2\) from U937 cells stimulated by concanavalin A (53). This activity is markedly decreased in homogenates of lungs and cells from Prdx6 null mice, confirming its association with Prdx6 (9).

The fifth line of evidence for Prdx6-PL\(A_2\) activity is the physiologic effects that are associated with genetic manipulation of Prdx6-PL\(A_2\) expression in mice (9, 38). These effects, discussed in detail below, include alterations of lung phospholipid turnover, altered cell membrane repair in oxidative stress, and failure of NADPH oxidase type 2 (NOX2) activation. Specificity for the role of the PL\(A_2\) activity of Prdx6 in the altered physiology has been confirmed by specific mutation of the PL\(A_2\) active site in Prdx6 (2, 10, 54–56).

**Tissue and cellular/subcellular distribution of Prdx6**

Prdx6 is a widely distributed protein that is expressed throughout the plant and animal kingdoms. In mammals, Prdx6 is expressed in virtually all organs, with the highest expression levels in lungs, brain, kidneys, and testes (26, 57). At the cellular level, the highest expression within the lung is in epithelium and alveolar macrophages with lower, but significant, expression in microvascular endothelial cells (26, 58). Within cells, the protein is largely cytosolic, but it is also expressed in acidic organelles [lysosomes and lysosomal-related organelles (LROs)] (27, 38, 59–61), as shown in Fig. 1. Prdx-PL\(A_2\) specific activity is nearly 3-fold greater in lung LROs [lamellar bodies (LBs)] as compared with lung homogenate (38). The cellular and subcellular localization of Prdx6 is important for understanding the physiologic role of its PL\(A_2\) activity. Prdx6 targeting to acidic organelles in lung epithelial cells is independent of lipid binding but requires a peptide sequence (amino acids...
For binding to the 14-3-3 protein as a chaperone; the chaperone function of 14-3-3 requires its phosphorylation by MAPK (59, 62, 63). Mutation of Ser32 in the targeting sequence abolishes Prdx6 binding to 14-3-3 and its targeting to LB (62).

**Structural basis for multiple enzymatic activities of Prdx6**

What special properties of Prdx6 allow it to express two essentially unrelated (peroxidase and PLA2) activities? We have proposed, based on the published X-ray crystal structures of the oxidized and reduced protein [Protein Data Bank (PDB) ID codes 1PRX and 2V2G] (64, 65), that the mode for binding of substrate to Prdx6 results in its positioning for either reduction at the peroxidatic site (amino acids surrounding C47 in Prdx6) or for hydrolysis by the PLA2 catalytic triad (S32-H26-D140) as shown in Fig. 2 (6, 7). The peroxidatic site is at the base of a shallow pocket, while the catalytic triad for hydrolysis is at the protein surface. The distance between Ser32 in the catalytic triad and the peroxidatic Cys47 in the pocket is ~28 Å, and there are a number of positively charged residues forming a groove between the two active sites (65). The key feature of the protein from an evolutionary standpoint is the development of a structure that allows binding of the lipid with access to these two distinct enzymatically active sites in the protein. Substrate binding by Prdx6 is discussed further below.

**Classification of Prdx6-PLA2 within the PLA2 family**

PLA2 represents a large family of enzymes that have been subclassified into 16 groups that largely reflect the timeline of their discovery (11–14, 66). Another classification, based essentially on protein localization and Ca²⁺ requirement, recognizes just five “types” of PLA2 enzyme (67). These include: type 1, secreted (extracellular) soluble enzymes (e.g., pancreatic PLA2) (68); type 2, extracellular enzymes associated with lipid-related proteins [e.g., platelet-activating factor acetyl hydrolase (PAFAH)] (44); type 3, intracellular calcium-dependent enzymes (e.g., cytosolic PLA2 and cPLA2) (69); type 4, intracellular calcium-independent enzymes (e.g., iPLA2) (70–72); and type 5, intracellular
enzymes present in acidic organelles (e.g., LPLA2) (45, 73). From a structure-function standpoint, type 1 (secreted) enzymes utilize a His-Asp catalytic dyad and are dependent on relatively high (millimolar) Ca\(^{2+}\) for activity (68); intracellular cytosolic (type 3, cPLA2) and Ca\(^{2+}\)-independent (type 4, iPLA2) PLA2 enzymes utilize a Ser-Asp dyad for activity—type 3 requires a relatively low (micromolar) Ca\(^{2+}\) for binding to substrate but not for activity, while type 4 PLA2 activity (obviously) is independent of Ca\(^{2+}\) (69, 74); and types 2 and 5 enzymes utilize a Ser-Asp-His (SDH) catalytic triad that also is independent of Ca\(^{2+}\) (44, 45). A recently described PLA2 activity that is localized to adipocytes uses a His-Cys dyad for its catalytic activity (12).

So, where does Prdx6-PLA2 fit in? Prdx6, when first isolated, was described as a LPLA2 based on the results of subcellular fractionation showing lysosomal and LRO localization and an acidic pH requirement for activity (9, 24–27, 50, 61). This, together with its calcium independence, led to the designation “acidic Ca\(^{2+}\)-independent PLA2” (aiPLA2) (24, 25, 53). Although PLA2 activities that were maximal at acidic pH had been described previously (71, 75, 76), aiPLA2 was the first such enzyme for which the molecular sequence was demonstrated (25). The PLA2 activity of Prdx6 is based on an SDH catalytic triad (6), and Prdx6 was one of the earliest PLA2 enzymes to be described with serine at its catalytic center (25). Subsequently, the molecular sequence of LPLA2 was identified as a second LPLA2 (73); identification of a lysosomal phosphatidylglycerol-specific PLA2 (77) probably represents parallel isolation of LPLA2 (46). Although aiPLA2 and LPLA2 both hydrolyze the \(sn-2\) fatty acyl linkage in phospholipids, many other characteristics, including size, inhibitors, and associated activities, are very different, as shown in Table 1.

A major difference between the two LPLA2 enzymes (aiPLA2 and LPLA2) is that Prdx6, although located in lysosomes and LROs, also is present in the cytosol; actually, the percentage of cellular aiPLA2 content is greater in cytosol than in acidic organelles, although its concentration is greater in the latter (24, 27). Furthermore, its activity, while maximal at acidic pH under basal conditions, is also present at neutral pH with oxidized phospholipid as the substrate or with reduced substrate following phosphorylation of the enzyme (see below). Thus, cytosolic Prdx6 has PLA2 activity toward cell membranes and can function as a PLA2 in both cytosolic (neutral pH) as well as lysosomal (acidic pH) compartments. Although LPLA2 can hydrolyze oxidized truncated phosphatidylcholine (PC), but not PC, at neutral pH, this activity is much less than activity at pH 4 (42).] The appellation aiPLA2, then, does not fully describe the enzymatic activity of the protein, although it does represent a convenient means to refer to the unique PLA2 activity associated with Prdx6. Thus, aiPLA2 represents a “hybrid” with characteristics of both intracellular Ca\(^{2+}\)-independent and LPLA2 types, as well as some characteristics not shared by either the type 4 or 5 enzymes. For now, aiPLA2 might reasonably be classified with the type 4 intracellular Ca\(^{2+}\)-independent PLA2 enzymes pending future insights leading to a more rigorous reclassification of this large and diverse group of proteins. An important caveat related to this classification is that aiPLA2 activity is not sensitive to bromoenol lactone (BEL) (24, 27, 54), an inhibitor of other type 4 PLA2 enzymes.
PLA2 Activity of Prdx6

Assay for Prdx6-PLA2 Activity

We have routinely assayed the PLA2 activity of Prdx6 based on the release of radiolabeled palmitate (either 9,10-3H-palmitate or 1-14C-palmitate) from the sn-2 position of dipalmitoyl phosphatidylcholine (DPPC) (23, 24, 27, 50, 54). In our assay, the substrate is presented as unilamellar liposomes containing labeled DPPC, egg PC, cholesterol, and phosphatidylglycerol (PG) in molar ratio 50:25:15:10, but use of a simpler liposome formulation (e.g., omission of PG, PS, PA, PE) certainly would be reasonable. Incubation is greater activity when assayed at pH 4 (100–130 nmol/min/mg protein) than at pH 7–8 (activity 0–50 nmol/min/mg protein), but considerably increased at pH 4 (100–130 nmol/min/mg protein) (54, 60). Although several “kits” are commercially available for assay of various PLA2 activities, there is no kit available, to our knowledge, with demonstrated specificity for aiPLA2. We have used a fluorescence assay with bis-BODIPY-C11-PC as the fluorophore substituting for radiolabeled phosphatidylcholine (25, 27), but key issues have been stability of the substrate under the acidic conditions required for optimal measurement of activity for the nonphosphorylated protein and the high background fluorescence for the manufactured substrate.

Substrate Specificity

aiPLA2 activity is greatest with PC as substrate; activity is decreased ~40% with the substitution of phosphatidylethanolamine, decreased another 60% with phosphatidylglycerol, and further decreased with other head groups (inositol and serine) as the PL substrate (27). The Km for the aiPLA2 reaction with PC as substrate is 350 µM (27). Activity of aiPLA2 toward PC shows no preference for the sn-2 acyl group, but is decreased substantially (~60%) with substitution of an alkyl linkage; however, there is no activity with 1-O-hexadecyl-2-acetyl-sn-glycerophosphocholine (platelet activating factor), i.e., no PAFAH activity (27). The protein also does not express PLA1 or lysophospholipase activities, but does express LPCAT activity (see below). Activities of recombinant protein based on the human or rat amino acid sequence or of protein isolated from either rat or bovine lungs are essentially similar (4, 27).

Effect of the Oxidation of Substrate or Enzyme

Oxidized phospholipid [oxidized sn-2 linoleic or arachidonic acid (AA)] is a substrate for aiPLA2 (8, 27). The activity at acidic pH is similar for reduced and oxidized substrate. However, in contrast to the reaction with reduced substrate, where activity is significantly decreased at pH 7 as compared with pH 4, PLA2 activity with oxidized substrate is similar at acidic and neutral pH (7, 27, 56). This gain of aiPLA2 activity at cytosolic pH with oxidized lipids as substrate is important for understanding the role of this enzyme in the repair of peroxidized cell membranes [Fig. 3]. Similar to this effect of oxidized substrate, PLA2 activity of Prdx6 at neutral pH is increased by oxidation of the protein (e.g., by treatment with H2O2), while activity at pH 4 is unaffected (unpublished observations from our laboratory and ref. 78). A possible (but untested) mechanism for this effect is that formation of the sulfenic acid intermediate associated with Prdx6 oxidation facilitates its binding to substrate. That is, the interactions of reduced Prdx6 with oxidized substrate or oxidized Prdx6 with reduced substrate both enhance protein-substrate binding and thereby enhance enzymatic activity.

1. The addition of GSH to the assay mixture in vitro also has a positive influence on the measured aiPLA2 activity at neutral pH (60). This effect is specific for GSH because addition of GSSG or several other (nonreducing) sulfhydryls had no effect (unpublished observations). Although not known precisely, we propose that the mechanism for this effect of GSH might be (paradoxically) oxidation of Prdx6 protein. Recombinant Prdx6 can be stabilized during storage by spontaneous formation of a sulfenylamide (5),
presumably by interaction of the sulfenic intermediate at C47 with an adjacent amino acid in the 3D structure; analysis of the crystal structure of the sulfonic acid form of Prdx6 suggests that the interacting amino acid may be His39 (65). Sulfenylamide formation may protect the protein against irreversible oxidation. By mass spectroscopy, this amide linkage is “broken” by addition of GSH, allowing autooxidation of the protein to the sulfonic form (unpublished observations). Thus, oxidation of the protein by treatment either with an oxidant (H2O2) or with GSH can result in increased aiPLA2 activity in vitro.

**Prdx6 phosphorylation**

While increased PLA2 activity is associated with oxidation of either the substrate or the protein, phosphorylation of Prdx6 (at T177) has a much greater effect on activity. PLA2 activity of phosphorylated Prdx6 was 1.3–1.7 µmol/min/mg protein at both acidic and neutral pH (36, 39, 79); these results were generated with protein that was less than fully phosphorylated so that the PLA2 activity of phosphorylated Prdx6 could be ~2 µmol/min/mg protein. Thus, phosphorylation of Prdx6 increases its PLA2 activity by 15- to 20-fold at pH 4 and (because of the lower baseline) to a much greater extent at pH 7 (35, 36, 39, 79). Physiologically, phosphorylation of cytosolic Prdx6 can occur through MAPK (ERK or p38) activity (54). The increase in PLA2 activity with phosphorylation reflects a conformational change in the protein as determined by tryptophan fluorescence and NaI fluorescence quenching (39). We have postulated that phosphorylation converts the protein to a less rigid molten globular state, allowing the conformational change (39).

**Substrate binding and aiPLA2 activity**

Physiologic activation of aiPLA2 activity requires the binding of Prdx6 to its substrate as an initial event, and analysis of the binding of this enzyme to substrate provides the basis for understanding PLA2 activity under various intracellular conditions (7, 80). Binding of the enzyme to the surface of the phospholipid vesicle (the substrate) is the first step for enzymatic activity, and conditions that promote binding are reflected in increased enzymatic activity (81). The isoelectric point for Prdx6, calculated as well as measured, is ~5.7–6.0 (82, 83), indicating that Prdx6 has a positive bulk charge under acidic conditions. Thus, Prdx6 binding to liposomal substrate is significantly increased by the negative charge imparted to liposomes with the inclusion of PG or phosphatidyserine in the lipid mixture (6). By ultrafiltration analysis, significant binding of protein to the negatively charged liposomes was demonstrated following incubation at pH 4, but binding was reduced markedly with incubation at pH 7 (7). Studies with protein mutagenesis have shown that the surface amino acid sequence H26-W31-S32 of Prdx6 is the site for phospholipid binding to Prdx6 (6). The increased binding at acidic pH presumably reflects protonation of a surface amino acid, most likely His26.

A recent crystallographic study (PDB ID code 2V2G) indicated that the distance between the H26 and D140 residues in Prdx6 is relatively great (~9.7 Å) and suggested that a phospholipid substrate actually may bind to the flat surface across the PLA2 active site and peroxidatic active site of the other monomer in the normal dimeric configuration of the protein (65). However, the experimental demonstration of PLA2 activity in the Prdx6 monomer indicates that dimerization of the protein is not necessary for activity (5). Furthermore, zero-length chemical cross-linking and homology modeling studies have shown that several regions of reduced human Prdx6 are in a substantially different conformation from that shown for the crystal structure of the peroxidase catalytic intermediate (84). Study of the catalytic triad in some other proteins also has shown considerable mobility of the active-site Ser residue (85). In support of plasticity of the Prdx6 protein, considerable conformational change has been demonstrated with

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**Fig. 3.** Reaction sequence for cell membrane lipid peroxidation and its repair by Prdx6. 1) Prdx6 is a cytosolic protein that does not bind to normal cell membranes. 2) Peroxidation of cell membrane phospholipid results in increased hydrophilicity of the oxidized (usually a sn-2) fatty acid. 3) Prdx6 binds to the oxidized cell membrane phospholipid. 4) Prdx6 can “repair” the peroxidized phospholipid by either: a) hydrolysis through aiPLA2 activity followed by reacylation through LPCAT activity, or b) reduction through PHGPx followed by alcohol dehydrogenase activities. 5) Prdx6 dissociates from the plasma membrane once the peroxidized membrane has been returned to the nonoxidized state. Modified from ref. 7 with permission.
its phosphorylation (39). Additional crystallographic studies of native Prdx6 that is bound to substrate under acidic conditions or to phosphorylated protein would help to clarify the binding paradigm.

While the binding of Prdx6 to phospholipid under acidic conditions is clear, a major fraction of intracellular Prdx6 is localized to the cytosol (25, 27, 59), where the neutral pH supports neither aiPLA2 binding to membranes nor its enzymatic activity under “resting” conditions. However, the cytosolic protein does bind to oxidized substrate at pH 7 so that oxidation of cell membrane phospholipids leads to cytosolic protein (39). The constant ($K_i$) for Prdx6 binding to substrate, estimated from fluorescence measurements, is 5.6 µM for phosphorylated protein compared with 24.9 µM for the nonphosphorylated WT (39). Phosphorylation changes the conformation of Prdx6 from its native state, resulting in the exposure of hydrophobic residues; the change in conformation results in a significant increase in PLA2 activity at both neutral and acidic pH (39).

**Inhibitors of aiPLA2 activity**

aiPLA2 activity is insensitive to most agents that are commonly used to inhibit other PLA2 enzymes, such as p-bromophenacyl bromide, arachidonoyl trifluoromethyl ketone, and BEL (25, 27, 38, 54, 56, 79). However, several effective inhibitors of aiPLA2 activity have been identified. Because aiPLA2 activity is serine-dependent, the enzyme is inhibited nonspecifically by the serine “protease” inhibitor, diethyl p-nitrophenyl phosphate (DENP) (4, 27). Another potent and more specific inhibitor of aiPLA2 activity is MJ33 (usually as the Li+ salt) (9, 23–25, 52, 86). This competitive inhibitor is an analog of the phospholipid tetrahydroxyl transition state that specifically interferes with the catalytic turnover by PLA2 enzymes that are bound to the enzyme-substrate interface (51). Inhibition of aiPLA2 activity is >90% at an inhibitor to substrate concentration of 1 mol% (24, 52). MJ33 also inhibits pancreatic PLA2 and several other PLA2 enzymes, although the affinity of the inhibitor for these other enzymes appears to be less than for Prdx6 (51).

We also have identified two endogenous proteins that inhibit aiPLA2 activity and might play a role in its physiologic regulation. The first such inhibitor is lung surfactant protein A (SP-A), a multimeric protein (26 kDa monomer) that is synthesized and secreted by lung epithelial cells as a component of lung surfactant (87). SP-A was shown to inhibit aiPLA2 activity and might play a role in the peroxidase activity of Prdx6, clearly showing that these two activities of Prdx6 are independent.

**Mutations of Prdx6 for the study of its PLA2 activity**

Several mutations of Prdx6 have been shown to be useful for the evaluation of its enzymatic and binding activities: 1) S32A or H26A mutations prevent binding of the protein to phospholipids, thereby abolishing PLA2 (and phospholipid hydroperoxide peroxidase) activities; peroxidase activity with small peroxides such as H$_2$O$_2$ is unaffected (6, 10, 55). 2) S32T mutation has no effect on Prdx6 binding to phospholipids or its enzymatic activities (Thr can substitute for Ser), but binding to 14-3-3ε is abolished, resulting in the failure of Prdx6 trafficking to acidic organelles (62). 3) Mutation of the D140A component of the catalytic triad abolishes PLA2 activity; there is no effect of this mutation on phospholipid binding or on other Prdx6-related activities (6, 56). 4) C47S mutation results in the total loss of peroxidase activity (phospholipid hydroperoxides and H$_2$O$_2$) but no effect on PLA2 activity (4, 10, 56, 58). 5) T177A mutation to Ala or Glu prevents Prdx6 phosphorylation and the subsequent increase in aiPLA2 activity; mutation to Glu results in increased basal aiPLA2 activity (36). 6) D31A mutation abolishes LPCAT activity, but there is no effect on aiPLA2 or peroxidase activities (10). 7) The double mutation L145/L148 prevents dimerization and results in loss of peroxidase activity, but PLA2 activity is intact; a single mutation of either Leu has an intermediate effect (5).

Summary of the reported mutations for study of specific physiologic effects of Prdx6: C47S eliminates all peroxidase activities; D140A eliminates PLA2 activity; D31A eliminates LPCAT activity; T177A prevents protein phosphorylation; L145A/L148A prevents protein dimerization; S92A prevents lipid binding; and S32T allows lipid binding but prevents the transport of Prdx6 to acidic organelles. It is important to emphasize that the frequently used S92A mutation of Prdx6 does abolish its PLA2 activity, but, through its effects on lipid binding, also abolishes PHGPx activity; this mutated protein expresses peroxidase activity for H$_2$O$_2$ and other small hydroperoxides, but has no activity toward the perhaps more important phospholipid hydroperoxide substrate. Thus, the loss of a physiological
PHYSIOLOGICAL ROLES OF PRDX6-PLA2 ACTIVITY

Regulation of lung phospholipid turnover

Lung surfactant, a complex lipid-protein product that is essential for mammalian life, promotes a low surface tension at the air-fluid interface of the lung alveoli, thereby minimizing the "work" of breathing and facilitating uniform lung ventilation (90). PC is the major biochemical component of the lung surfactant, comprising approximately 75% of the secreted product; approximately 2/3 of PC is the dipalmitoyl form (DPPC) that functions as the primary surface-active material (52, 91). Lung surfactant has a complex life cycle, as it is synthesized by alveolar granular pneumocytes (epithelial type 2 cells), stored in the LBs of the pneumocytes, secreted into the alveolar space by exocytosis, taken up by the same cells through receptor-mediated endocytosis, and reprocessed in LB for degradation or resecretion (52, 92–94). The measured half-time for DPPC in the alveolar space has been estimated, based on the label in palmitate, at approximately 10 h for small animal models and at 28 h for primates (91, 92). Extracellular (alveolar) DPPC that is recycled to LBs is degraded and/or remodeled prior to resecretion (92). LB are LROs that maintain an acidic pH (approximately pH 5) (95) and thus can support the PLA2 activity of Prdx6. The physiological “purpose” of this recycling pathway is not understood but is related most likely to quality and/or quantity control of the lung surfactant material.

Prdx6-PLA2 activity is involved in both the degradation and the remodeling of surfactant phospholipids (9, 23, 24, 38, 50, 52). Degradation of DPPC involves PLA2 activity as an initial step with subsequent metabolism of the liberated palmitate and lysoPC fractions. Remodeling is accomplished through PLA2 activity to generate lysoPC, followed by reacylation with an acylCoA, catalyzed by LPCAT, to regenerate PC; the remodeling reactions represent the classical Lands cycle for PC synthesis (94). Because Prdx6 expresses both PLA2 and LPCAT activities, it is, therefore, a complete remodeling enzyme.

DPPC in lung surfactant also can be catabolized by alveolar macrophages (96). This pathway represents a scavenging pathway for removal of “damaged” or excessive phospholipids and is not part of the elegant surfactant recycling mechanism. Uptake of phospholipids by macrophages for degradation is through phagocytosis rather than the receptor-mediated pathway for recycling. As Prdx6 is expressed at a significant level in alveolar macrophages (54), αiPLA2 could play a role in this degradative (scavenging) pathway, although that has not yet been studied. LPLA2 also is expressed in alveolar macrophages, and this, rather than αiPLA2, may be the major macrophage-related phospholipid degradative enzyme (97, 98).

The phenotype of Prdx6 null lungs demonstrates the role of αiPLA2 activity in phospholipid homeostasis (9). In mice aged 9–11 weeks, the PC content of Prdx6 null compared with WT control was increased by 73% in the lung, 29% in the lung LB, and 58% in lung broncho-alveolar lavage fluid (BALF). Unlike lungs from WT mice where the PC content normalized to lung protein or body weight remains relatively constant with time, lungs from Prdx6 null mice show a linear increase in lung PC, lung DPPC, and lung total phospholipid; at 1 year of age, PC in null mice compared with control is greater by 200% in the lung and 180% in the BALF. In experiments to evaluate the metabolism of lipids, lungs from WT mice degraded 54% of internalized DPPC over a 2 h observation period, while lungs from Prdx6 null mice degraded only 5%. To study PL synthesis, mice were injected with radiolabeled palmitate into the tail vein; when studied 24 h later, there was 73% less radioactivity in LB PL from null lungs as compared with WT (9).

WT lung alveolar type II epithelial cells in primary culture showed a marked decrease in PC synthesis following their treatment with MJ33, similar to that seen in Prdx6 null lungs (24). Inhibition of αiPLA2 activity prevented phospholipid remodeling with mechanical stress with the lung cell line A549 in culture (99). For the opposite effect, transgenic Prdx6 overexpressing mice showed a 17–25% decrease in PC content of lungs, LBs, and BALF and a 36% increase in the degradation of radiolabeled DPPC that had been administered to lungs and internalized by lung cells (38). Thus, Prdx6 plays an important role in the turnover of lung surfactant phospholipids.

Antioxidant defense and cell membrane repair

Exposure of cells to oxidants [termed reactive oxygen species (ROS)] results from normal metabolism as well as in association with toxic exposures. Organisms express a variety of antioxidant enzymes, including several peroxidases, to counter oxidant “stress.” Thus, the GPx activity of Prdx6 as a scavenger of H2O2 and hydroperoxides has received attention as an antioxidant enzyme (100, 101). However, the PLPLA2 activity of Prdx6 also plays a major role in antioxidant defense related to its role in the “repair” of oxidized cell membranes (2, 55, 56, 58). Because the repair of oxidized cell membrane phospholipids represents the reversal of the effects of oxidative stress, it can be considered as an antioxidant activity.

An important role for PLPLA2 activity in the repair of oxidized cell membranes was suggested some time ago (102, 103), but the specific PLPLA2 involved was not known and identification of αiPLA2 as specific for these reactions is relatively recent. WT lungs and endothelial cells show repair of oxidized cell membrane phospholipids over several hours following removal of the oxidant (Fig. 4); however, Prdx6 null lungs that express neither the peroxidase
nor the PLAD2 activities of Prdx6 fail to show significant cell membrane repair following a lipid peroxidation event (7, 56, 58). Based on the high rate constant (K2 > 10^6/M/s) for the phospholipid hydroperoxide reductase (19), we had assumed based on theoretical calculations (104) that this reaction would predominate in peroxidized cell membrane repair. However, experimentally, that has proved not to be the case, and specific mutations of Prdx6 that abolish either the peroxidase or the PLAD2 activity of the protein have indicated that the aiPLAD2 activity of Prdx6 can contribute approximately equally to the PHGPX activity for the maximal rate of cell membrane repair (55, 56, 58).

While the peroxidase activity of Prdx6 directly reduces a peroxidized membrane phospholipid (to its corresponding alcohol), the Prdx6-PLA2 activity liberates the oxidized fatty acid by the hydrolysis of the sn2 acyl bond; the lysoPC that is generated by this reaction can be reacylated through the LPCAT activity of Prdx6 (10). Based on kinetic studies, the sequential deacylation and reacylation reactions for Prdx6 occur without release of lysoPC from attachment to the enzyme (10). Thus, these linked activities of Prdx6 (PLA2, LPCAT) can replace the oxidized fatty acid of a phospholipid with a nonoxidized one, thereby regenerating the reduced phospholipid.

This repair function linked to Prdx6-PLA2 activity has been demonstrated for acute oxidative stress, but it is possible that the membrane repair function of Prdx6 also plays an important physiological role under chronic conditions. For example, decreased reproductive efficacy has been demonstrated in aging mice and is accelerated by the absence of Prdx6-PLA2 activity (105, 106) (see below). This finding may reflect a role for Prdx6 in protection against the chronic effects of cell membrane lipid peroxidation.

Cell signaling: activation of NOX2

NOX2 is a multicomponent enzyme that is expressed by professional phagocytes (polymorphonuclear leukocytes, or PMN, macrophages), microvascular endothelium, and other cell types. This enzyme complex has the primary function to generate the superoxide anion that plays an important role in bacterial killing as well as in normal cell signaling related to cell proliferation, cell migration, immune function, aging, stem cell self-renewal, and other homeostatic processes (107). NOX2 itself is activated by a signaling pathway that has long been proposed to require PLA2 activity, although neither the specific PLA2 nor the relevant product were known (108). Initially, the enzyme called group IV (cytosolic) PLA2 was considered for this role (109), but this is unlikely based on studies with cPLA2 null mice (110). More recent studies have demonstrated that Prdx6 PLA2 activity is required for NOX2 activation, at least in PMN, macrophages, and lung microvascular endothelial cells (54, 111). Following an activating signal, Prdx6 is phosphorylated and “translocates” to the plasma membrane (54); this translocation reflects the marked increase in the ability of Prdx6 following its phosphorylation to bind to phospholipids in membranes (7). Inhibition or “knock out” of Prdx6-PLA2 activity essentially abolishes NOX2 activation in response to activating stimuli (54). The mechanism for the role of Prdx6 in NOX2 activation is through aiPLA2-mediated generation of lysoPC that, in turn, is converted to lysosphosphatidic acid (LPA); LPA reacts with a cell membrane receptor (LPA receptor 1), leading to liberation of Rac (112), a crucial component of the NOX2 activation pathway (113).

As described above, p67phox binds to phosphorylated Prdx6 and inhibits its PLAD2 activity (35), or, conversely and not yet tested, binding of phosphorylated Prdx6 to p67phox may inhibit the phosphorylation of the latter protein. Further study is required to determine whether this interaction constitutes a physiological mechanism for regulating NOX2 activity.

An example of the physiological role of activated NOX2 is the response of pulmonary microvascular endothelium to altered mechanical stress; these cells in culture on going from a flow to no-flow condition (simulated ischemia) show a brisk aiPLA2-dependent generation of ROS that has been associated with endothelial cell growth, cell proliferation, and vascular remodeling, among other effects (114). Activation of NOX2 by aiPLA2 activity also has a major role in the inactivation of a G-protein-coupled opioid receptor (115).

In addition to NOX2, Prdx6 has been shown to facilitate the activation of NADPH oxidase type 1 (NOX1) (116). Prdx6 can bind to and stabilize the expression level of NOX1, a homolog of p67phox and a cytosolic activating protein for NOX1. Activation of NOX1 was not seen in the presence of S32A mutant Prdx6, reflecting either the loss of aiPLA2 activity or the failure of lipid binding by the
The role of aiPLA2 activity to stabilize the expression levels of the cofactor was suggested by MJ33-mediated inhibition of the effect (116); however, the mechanism for this effect of aiPLA2 activity on NOX1 is unclear. Because Rac appears to be an important cofactor for NOX1 activation (117), a possible mechanism for the involvement of Prdx6 in NOX1 activation is the generation of active Rac through aiPLA2-generated lysoPC, as for NOX2. That possibility has not been tested.

The role of aiPLA2 in the activation of NOXs indicates that Prdx6 can function either as a prooxidant or an antioxidant enzyme. Prdx6 does indeed appear to serve both functions. On the one hand, Prdx6 is crucial for the activation of NOX2, a major physiological source of the oxidant superoxide anion that plays an essential role in cell signaling; on the other hand, the peroxidase activity of Prdx6 can scavenge ROS (H2O2), while its ability to reverse membrane oxidation through its peroxidase as well as PLA2 activities can prevent the consequences of oxygen radical overproduction. Thus, Prdx6 can be regarded as an enzyme to “normalize” the intracellular content of ROS; that is, it is involved in the generation of ROS for cellular signaling, but also in decreasing excessive cellular H2O2 levels and in protection against the side effects of ROS overproduction.

**aiPLA2 IN PATHOPHYSIOLOGY**

A role for Prdx6 has been described for a wide range of both human pathologies as well as for animal models of disease. For the most part, evidence for the role of Prdx6 is based on the finding of an increased or a decreased expression level for the protein, occurring either as a result of the disease or through genetic manipulation. In some instances, an altered Prdx6 expression level may merely reflect the role of inflammation that leads to induction of Prdx6 along with other antioxidant enzymes. Thus, there currently are relatively few well-documented examples where expression of Prdx6 plays a specific role in the pathophysiology of disease, and fewer still where there is an apparent role specifically for Prdx6-PLA2 activity.

**Acute lung injury and sepsis**

Acute lung injury (ALI; or acute respiratory distress syndrome) represents either a final common pathway for many etiologies that primarily affect the lung or a secondary response to systemic diseases (118). Pneumonia, either bacterial or viral, is the most common primary lung disease resulting in this syndrome, while sepsis is a common cause of secondary ALI. Hallmarks of ALI include lung inflammation associated with an influx of PMN and increased generation of ROS, in large part through activation of the enzyme NOX2 in PMN, alveolar and tissue macrophages, lung endothelium, and possibly other cell types (118). Excessive ROS production can lead to tissue injury through oxidation of cellular proteins and lipid peroxidation of cell membranes.

Prdx6-PLA2 activity can play two major roles in ALI. First, through its role in the activation of NOX2, aiPLA2 can contribute to the pathophysiology of lung damage in ALI (54). Second, through its effects on cell membrane phospholipids, aiPLA2 participates in the repair of oxidative injury (56). Although the inhibition of aiPLA2 activity may reduce the ability of Prdx6 to repair peroxidized cell membranes, repair through reduction of phospholipid hydroperoxides, i.e., the PHGPx activity of Prdx6, would be unaffected. Thus, inhibition of aiPLA2 activity by administration of MJ33 led to a marked reduction of lung injury including the extent of lipid peroxidation in mouse models of ALI associated with the intratracheal administration of bacterial lipopolysaccharide, ischemia-reperfusion injury, or exposure to hyperoxia (19, 86, 119, 120).

**Cancer and carcinogenesis**

Elevated expression levels for Prdx6 have been demonstrated in association with a broad variety of human cancers, including lymphoma and cancer of the breast, esophagus, lung, liver, ovaries, pancreas, bladder, thyroid, gingiva, tongue, skin, and mesothelium, among others (121–133). Increased expression of Prdx6 correlated with a poorer prognosis for breast cancer and large B-cell lymphoma, progression of bladder cancer, and increased invasiveness of retinoblastomas (124, 135–136), but correlated with improved survival of patients with hepatocellular carcinoma (123) and some types of pancreatic adenocarcinoma (136). The correlation of Prdx6 expression with poor prognosis for breast cancer was associated with several SNPs of Prdx6, although these SNPs were unrelated to the active site for aiPLA2 activity (124). This effect of Prdx6 expression was supported by studies with human breast cancer cells in vitro showing increased proliferation and invasiveness associated with Prdx6 overexpression (125). Similarly, overexpression of Prdx6 in lung and gastric cancer cells in vitro led to an increased rate of tumor cell growth, cell migration, and cellular invasion (126–128, 132). These adverse effects of Prdx6 in the gastric cancer cell line were reversed by treatment with a microRNA (miR-24-3p) (128). To briefly summarize these studies of human cancers and cancer cells, the increased expression of Prdx6 in cancers has frequently been associated with increased invasiveness and a poorer prognosis, although the opposite effect also has been reported.

As for human tumors, Prdx6 overexpression increased the growth and invasiveness of established experimental tumors in virally and chemically induced mouse skin carcinoma; however, the presence of Prdx6 inhibited the formation of new tumors (132). Based on the correlation of tumor number with lipid peroxidation in the skin carcinoma model, increased susceptibility to tumor formation could reflect a low-level chronic inflammation (132), although that remains open to further investigation. Assuming that chronic low-level lipid peroxidation does support carcinogenesis, both aiPLA2 and peroxidase activities of Prdx6 would be involved because both contribute to the repair of peroxidized cell membranes (56). Thus, Prdx6 might have a dual role in carcinogenesis by suppressing the...
formation of new tumors, but stimulating the growth of existing tumors.

On the other hand, a reasonable mechanism for increased tumor growth and invasiveness associated with Prdx6 overexpression has not been proposed. Prdx6 prevented hyperglycemia-mediated apoptosis in lens epithelial cells (137), and decreased apoptosis in tumors could support an increased rate of tumor growth. However, to the contrary, Prdx6 supported, rather than inhibited, TNFα-mediated apoptosis in hepatocellular carcinoma cells in vitro and also in primary bronchial epithelial cells and a gastric cell line (123, 128, 138). This latter effect would tend to dampen rather than increase tumor growth and invasiveness as observed for Prdx6 overexpression. Additional studies are clearly required to resolve these issues.

Several studies have specifically evaluated other roles of Prdx6 enzymatic activities in carcinogenesis. Knockdown of presenilin 2 resulted in increased tumor growth in a mouse model of lung carcinoma; this effect was attributed to a secondary increase in aiPLA2 activity, although the assay used to determine enzymatic activity was nonspecific (139). The studies described above relating Prdx6 to apoptosis in hepatocellular carcinoma cells indicated that its PLA2 activity was responsible for the effects (129); however, that conclusion is suspect because it was based on the use of an inhibitor, BEL, that does not inhibit aiPLA2 activity (26, 27, 54). In a study of lung cancer cells, mutations S32A and C47S in Prdx6 both abolished the effect of WT Prdx6 on tumor metastasis, while MJ33 treatment had no effect (130); although the authors interpreted these data to show involvement of both PLA2 and peroxidase activities, a more likely interpretation is that the phospholipid hydroperoxide peroxidase (PHGPx) activity of Prdx6, but not the PLA2 activity, is responsible for the results. In a study of melanoma cells, the suppression of cell growth by Prdx6 “knockdown” was rescued by transfection with a Prdx6 construct that expresses aiPLA2, but not peroxidase activity (129). The proposed mechanism for the activation of melanoma tumor growth was through AA release resulting from aiPLA2 activity (129); while that mechanism is feasible, recombinant Prdx6-PLA2 does not show specificity for AA-containing phospholipids (27). Additionally, as described above, mutation of S32, purportedly to confirm the role of PLA2 activity, also abolishes PHGPx activity, further confounding the results.

An increase in the number and size of urethane-induced adenocarcinomas and the growth of A549 and NCI-H460 human lung cancer cells in mice was attributed to activation of the JAK2/STAT3 pathway, but the deletion of either the GPx activity (C47S-Prdx6) or the aiPLA2 activity (S32A-Prdx6) of Prdx6 had no effect on phosphorylated (activated) JAK2/STAT3 expression in these cells (127, 140). Although the authors concluded that either enzymatic activity by itself could support activation of the pathway, this conclusion is suspect because either mutation (C47S or S32A) would have abolished PHGPx activity leaving as remaining activities only the hydrolysis of PC (through aiPLA2 activity of the C47 mutant) or the reduction of H2O2 or similar small oxidant (through GPx activity of the S32 mutant); it is unclear how either one of these activities alone could activate the JAK2/STAT3 pathway. Other activators proposed for the effect of aiPLA2 activity on tumor growth include (in different studies) MAP or Src family kinases (127, 129, 130), urokinase plasminogen activator or its receptor (125, 130), or various other signaling proteins (125), although definitive evidence for their possible role in aiPLA2 activation was not presented.

Based on these various studies, a preliminary conclusion is that aiPLA2 activity can support tumor growth, invasiveness, and metastasis. These effects of Prdx6 may vary with cell type. However, some studies present contradictory information. Further, the mechanism for these proposed effects of Prdx6 is unclear. Thus, additional and better controlled studies will be required to fully understand the importance and the mechanistic basis for the role of aiPLA2 in carcinogenesis.

Diseases of the CNS

Prdx6 overexpression has been demonstrated in a variety of CNS neurodegenerative diseases and related animal models including Parkinson’s disease (141), amyotrophic lateral sclerosis (142), Alzheimer’s disease (143), and several other types of dementia (141, 144, 145). Brains from patients with Alzheimer’s disease showed an increased number and increased staining intensity of Prdx6-positive astrocytes that were closely involved with diffuse plaques (143). Overexpression of Prdx6 protected against neuropathology in a mouse model of prion disease (146) and served as a biomarker of traumatic brain injury (147, 148). Prdx6 expression was markedly increased in the spinal cord of mice with experimental autoimmune encephalomyelitis, as a model of multiple sclerosis and transgenic overexpression of the protein in this experimental model resulted in a significant decrease in clinical severity (149). However, none of these studies specifically evaluated which activity of Prdx6 was responsible for the findings, and increased Prdx6 expression may have merely reflected an ongoing oxidant stress.

Several studies have attempted to evaluate the role of aiPLA2 activity in mouse models of CNS pathology. Overexpression of Prdx6 was shown to potentiate the neurodegeneration associated with administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a mouse model of Parkinson’s disease (150), and the CNS pathology in a mouse model of Alzheimer’s disease (151). Although the assays for Prdx6-PLA2 activity in these studies were not definitive, the administration of MJ33 provided evidence that the adverse effects of Prdx6 in these models might have reflected an increase in the aiPLA2 activity in specific brain cells.

Diabetes

Prdx6 null mice develop a phenotype similar to early stage human type 2 diabetes manifested by reduced glucose-dependent insulin secretion and increased insulin resistance (152). A role for the loss of aiPLA2 activity in the pathogenesis of these metabolic manifestations might be expected, but has not yet been tested.
Male infertility

The aiPLA2 activity of Prdx6 may play a role in male fertility. Spermatozoa from Prdx6 null mice have impaired fertilizing ability that is accentuated during aging (105, 106), and treatment of sperm from wild-type mice with MJ33 decreased their motility, viability, and fertilization rates (153). These results suggest that the decrease in fertility with aging in mice is related to decreased expression of aiPLA2 activity. The mechanism for these effects of aiPLA2 may reflect its role in ROS-mediated signaling that is required for the subsequent phosphorylation events that lead to sperm capacitation (154), although the effects of an increase in lipid peroxidation products also would be possible. As for the relevance of these results to humans, infertility in human males has been associated with decreased expression of Prdx6 (155). Furthermore, treatment of human sperm with MJ33 decreased their motility and fertilization ability that is accentuated during aging (105, 106), and treatment of sperm from wild-type mice with aiPLA2 activity has an important role in the male reproductive system. The role of changing aiPLA2 expression in the decreased fertility with human aging has not yet been studied.

Other pathophysiology

Altered expression of Prdx6 has been reported for several miscellaneous diseases. Lower Prdx6 expression correlated with a younger age for human cataract surgery while treatment with Prdx6 delayed cataract formation in animal models (157–159) and protected against cytotoxicity in retinal pericytes (137). Prdx6 content in blood serum was increased in pobaric hypoxia as a model for acute mountain sickness (138) and led to increased levels (160). Prdx6 activity has an important role in the male reproductive system. The role of changing aiPLA2 expression in the decreased fertility with human aging has not yet been studied.

SUMMARY AND CONCLUSIONS

In summary, Prdx6 is an intracellular Ca2+-independent PLA2 enzyme (called aiPLA2), and this protein also expresses GPx and LPCAT activities. aiPLA2 activity has an important role in several physiologic functions. These functions include the phospholipid metabolism of lung alveolar type 2 epithelial cells, although the role of this enzyme in the lipid metabolism of other organ and cell types has not yet been investigated. aiPLA2 activity also has a major physiological role in utilizing the remodeling pathway to repair peroxidized cell membrane phospholipids associated with oxidative stress. Additionally, the aiPLA2-mediated generation of lysoPC for conversion to lysoPA is important in the complex pathway for the activation of NOX2. Although aiPLA2 activity has been associated with a variety of diseases, including ALI and sepsis, cancer and carcinogenesis, degenerative CNS diseases, diabetes, and male infertility, among others, additional evidence is necessary for a full appreciation of its pathophysiologic significance.

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