Epigenetic regulation of peroxiredoxins: Implications in the pathogenesis of cancer

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Abstract

Peroxiredoxin I to VI (PRX I–VI), a family of highly conserved antioxidants, has been implicated in numerous diseases. There have been reports that PRXs are expressed aberrantly in a variety of tumors, implying that they could play an important role in carcinogenesis. Epigenetic mechanisms such as DNA methylation, histone modifications, and microRNAs have been reported to modulate expression of PRXs. In addition, the use of epigenetic regulators, such as histone deacetylases, has been demonstrated to restore PRX to normal levels, indicating that the reversible nature of epigenetics can be exploited for future treatments.

Keywords: Peroxiredoxin, epigenetic regulation, microRNA

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Introduction

The six members of the peroxiredoxin (PRX) family of highly conserved antioxidant enzymes, *viz.* PRX I to VI, can be found in all organisms across the animal kingdom.¹ PRX I–VI can be categorized into two groups, depending on how many conserved cysteine residues are present at the carboxyl and amino terminals: 1-Cys PRX and 2-Cys PRXs.^{1–3} 2-Cys PRXs, which contain a cysteine residue at both terminals, can be segregated into two subgroupings: typical 2-Cys PRXs and atypical 2-Cys PRX. Typical 2-Cys PRXs consist of PRX I–IV, while PRX V is the only atypical 2-Cys PRX. PRX VI belongs to 1-Cys PRX because of the presence of a conserved cysteine residue only at the amino terminal. The first step of all PRXs activity appears to be similar, in which redox active cysteine reacts with the peroxide substrate, giving rise to oxidized cysteine sulfenic acid. It is the next step of the catalytic reaction that distinguishes the different groups of PRXs. Typical 2-Cys PRXs are homodimers, in which the peroxidatic cysteine on one subunit, reacts with the resolving cysteine on the other subunit, to form a stable intermolecular disulfide bond.¹ Whereas atypical 2-Cys PRX is a monomer, and hence, the presence of a condensation reaction between peroxidatic cysteine and resolving cysteine creates an intramolecular disulfide bond.³ Lastly, 1-Cys PRX VI does not form a disulfide bond because a resolving cysteine residue is lacking at its carboxyl terminal.³

Physiological functions of PRXs

PRXs exert their antioxidative protective function by reducing and detoxifying a variety of reactive oxygen species (ROS) that includes, hydrogen peroxide (H₂O₂).⁴ The six isoforms exist in multiple subcellular compartments, each performing non-redundant functions. PRX III, localized specifically to the mitochondria, has been proposed as the first line of defense against ROS in this organelle.⁵ As PRX I is localized mainly to the cytosol and partly to the nucleus, it offers protection mainly for nucleic acids, lipids, proteins, and other macromolecules from the deleterious effects of ROS.³

H₂O₂ is being constantly produced by cells and its elimination is dependent on antioxidant enzymes that include catalases, glutathione peroxidases, and also the redox-active cysteines of PRXs.³ Typically, thioredoxin (TRX) is the preferred electron donor of oxidized PRXs and the resultant oxidized TRX is subsequently reduced and recycled by Trx reductase (TrxR) (Figure 1).⁶ PRXs becomes hyperoxidized when the sulfenic acid intermediate is overoxidized into sulfinic acid.⁷ This hyperoxidized form of PRXs was previously thought to be irreversibly oxidized, and hence terminating the enzymatic cycle of PRXs. However, it has been demonstrated that hyperoxidized PRX can be reversed to the catalytically active form, by sestrin 2 and sulfiredoxin (SRX) which utilizes ATP.^{8–10} Interestingly, hyperoxidized PRXs retain some reducing capacities⁶ and the newly formed structures have been

Figure 1 A schematic showing the generalized catalytic activity of PRXs. TRX: thioredoxin; TrxR: thioredoxin reductase; SRX: sulfiredoxin. (A color version of this figure is available in the online journal.)

proposed to signify a change in PRXs role as antioxidants to molecular chaperones during oxidative stress.^{6,11,12}

Modulation of H_2O_2 , a second messenger, enables PRXs to serve diverse roles in immunity response, apoptosis, cell proliferation, cell differentiation, and cell signaling.^{2,5,11} For H_2O_2 to function as a signal, it must be able to increase above a threshold rapidly and maintain the concentration for H_2O_2 to interact with its effectors. This notion, which has been coined the “floodgate” hypothesis,¹¹ describes the transient inactivation of PRXs by hyperoxidation which causes accumulation of H_2O_2 , and thus allowing signal transduction to occur.^{11,13}

Aberrant PRXs expression in diseases

Over the years, cumulative evidence has shown that oxidative damage plays essential roles in various disease processes, such as neurodegeneration, with frequent deregulation of PRXs in many disease states. Collectively, this seems to suggest that the PRXs could contribute to disease progression. Parkinson’s Disease (PD), Down Syndrome, and Alzheimer’s Disease are neurodegenerative diseases that are associated with ROS-induced damage, whereby PRXs expression levels were discovered to be altered in these disease states.¹⁴ Moreover, upregulation of PRX VI was reported to worsen dopaminergic neurodegeneration in mouse model of PD,¹⁵ thus suggesting that PRXs serve important role in maintaining ROS balance in the brain.

In carcinogenesis, oxidative stress and ROS contribute to disease progression predominantly by causing genomic instability and activation of signaling pathways involved in malignant transformation.¹⁶ Hence, antioxidants such as PRXs and GSHs which maintain redox balance are believed to play crucial roles in cancer pathogenesis. In fact, PRX I, III, IV, and V have been observed to be up-regulated in breast cancer, among the malignancies of various organs and tissues.¹⁷ One of the major hallmarks

of cancer is the ability to sustain constant proliferation¹⁸ and ROS have been implicated in the regulation of cell cycle progression.^{19,20} ROS regulate cell cycle progression by functioning as second messengers in signaling pathways,²⁰ ubiquitination and phosphorylation of cell cycle-associated proteins, and also activation of growth factor receptors independent of the ligands.²¹ In loss of function studies, depletion of PRX III has been reported to inhibit cell proliferation in breast cancer cells.²² Downregulation of PRX II has been demonstrated to inhibit cellular growth in colorectal cancer, by affecting the Wnt/ β -catenin signaling pathway.²³ PRX VI is known to facilitate cellular invasion and proliferation in lung and breast cancer.^{24–26} Moreover, PRX I was observed to interact with c-Myc and c-Abl, which are important regulators of cellular proliferation.²⁷

In addition to sustained cellular proliferation, cancer cells are able to evade apoptosis signals.¹⁸ ROS are known to take on a major role in triggering the onset of apoptosis, especially in the intrinsic pathway which is also known as the mitochondrial pathway.²⁸ ROS and oxidative mitochondrial damage facilitate the permeabilization of mitochondrial membrane, activation of Bax and Bad, and also the release of cytochrome c, which in turn activate downstream caspase-dependent and caspase-independent signaling pathways.²⁸ In the extrinsic apoptosis pathway, ligands bind and activate death receptors such as Fas and TNF receptor 1. These death receptors contain a death domain which is sufficient to trigger activation of caspases, leading to apoptosis.^{28–30} However, ROS appears to play a role in TNF-mediated apoptosis as treatment with N-acetylcysteine (an antioxidant) inhibited the onset of apoptosis.³¹ The mitochondria is a major site of ROS production and also the main mediator of apoptosis.³² Therefore, PRX III which is predominantly localized to the mitochondria, may significantly affect the apoptotic pathway. Indeed, studies have reported that PRX III knockdown lead to increased ROS levels, which was subsequently followed by enhanced apoptosis.^{5,33–35} Furthermore,

Figure 2 Pro-tumorigenic roles of PRXs. PRXs augment cell survival by decreasing apoptosis, promote proliferation, and potentiate metastases of cancer cells. PRXs contribute to therapy resistance by reducing oxidative stress. (A color version of this figure is available in the online journal.)

it was discovered that PRX III knockdown facilitated the release of cytochrome c and caspase activation, thereby promoting apoptosis.⁵ In addition, knockdown of cytosolic PRX II in A549 gefitinib-resistant cells (A549/GR) resulted in the restoration of ROS and apoptosis back to control levels.³⁶ Therefore, PRXs appear to have significant roles in the regulation of apoptosis in cancer cells.

Besides their biological effects on cellular proliferation and antiapoptotic functions, aberrant PRXs expression are also involved in the mechanisms of chemotherapy and radiation resistance. One such mechanism that radiation therapy and chemotherapy are dependent on, is the increase in intracellular ROS, thereby causing oxidative stress and eventually triggering the onset of apoptosis.³⁷ However, expression of PRX proteins has been observed to be elevated in various carcinomas,^{35,38} suggesting that excess PRXs function to overcome the increased ROS, thus enabling cancer cells to overcome elevated oxidative stress, thereby enhancing survival. Elevated level of PRX II was found to confer radiation resistance in cancers of the head and neck region³⁹ and breast cancer.⁴⁰ Both studies demonstrated that downregulation of PRX II improved radiation sensitivity in radiation-resistant cancer cells. Moreover, PRX I silencing by promoter methylation has been reported to increase chemotherapy and radiation sensitivity of Hs683 glioma cells *in vitro*.⁴¹ It would appear that downregulation of PRXs sensitizes chemo- and radio-resistant cells, thereby highlighting the importance of PRXs in radiation and drug sensitivity.

Cancer cells are also known to be able to migrate and invade tissues at distinct sites, giving rise to metastasis.¹⁸ ROS have been implicated in cellular migration due to its ability to activate signaling pathways, such as c-JUN NH-2 terminal kinase and p-38 MAPK.⁴² During invasion, cells secrete proteases such as matrix metalloproteinases (MMPs) and induce urokinase plasminogen activator, to degrade extracellular matrix, paving the way for cellular migration.⁴² ROS trigger signaling transduction pathways that up-regulate these proteases, leading to enhanced invasive potential of cancer cells.⁴³ Upregulation of PRX VI has been demonstrated to enhance invasiveness and metastatic potential of cancer cells, by modulating expressions of genes such as MMP-9 and TIMP-2.^{24,44}

Taken together, PRXs in cancer cells contribute to cancer progression by enhancing survival, proliferation, and metastatic ability (Figure 2). The PRX family of antioxidants also contributes to therapy resistance.

The importance of PRXs in carcinogenesis has led to the perception that PRXs are potential biomarkers. Overexpression of PRX III detected in hepatocellular carcinoma (HCC) has been associated with about 95% HCC, suggesting that PRX III may be an important biomarker of HCC.⁴⁵ Another study reported that serum PRX III expression was inversely correlated to survival in patients in early stage HCC, implying that expression of PRX III may allow for early diagnosis and predicting poor prognosis of HCC.⁴⁶ Moreover, PRX III was also proposed to function as a marker for cellular proliferation in breast cancer and

Figure 3 Epigenetic regulation of PRXs. (A color version of this figure is available in the online journal.)

cervical cancer.^{22,47} Furthermore, PRXs expression could potentially be a marker for radiation responsiveness, since elevated PRXs levels can be frequently observed in resistant cells. This allows for more precise selection of suitable patients that would benefit immensely from the therapeutic regimes to be instituted. In fact, a study pertaining to osteosarcoma showed that PRX II expression was found to be positively correlated with poor response to chemotherapy, suggesting that PRX II might be a potential chemotherapy responsiveness marker.⁴⁸

Regulation of PRXs via epigenetic mechanisms

Reversible hyperoxidation has been proposed as a form of regulation of PRXs, by temporarily inactivating their catalytic function.³ In addition, epigenetic mechanisms have also been reported to regulate expression of PRXs and these regulatory mechanisms could form the basis for potential new treatments of diseases. Epigenetics refer to heritable changes in gene expression patterns which cannot be detected from the DNA sequence.^{49–51} The main epigenetic mechanisms include DNA methylation, covalent modifications of histones, and microRNA (miRNA) (Figure 3). Interestingly, DNA and histone modifications not only modulate coding genes but miRNA as well. Conversely, miRNAs have been determined to regulate crucial epigenetic regulators such as histone deacetylases (HDACs).⁵² The interplay between these complicated pathways form the “epigenetic landscape” or epigenome of

an organism, which controls temporal and spatial gene expression in diverse cell types throughout developmental processes.^{49,53}

DNA methylation

DNA methylation is a form of covalent modification of DNA which results in stable gene silencing.⁵³ In mammals, a pattern known as global methylation is observed in which DNA methylation occurs on the cytosines of CpG dinucleotides that are dispersed throughout the genome.⁵⁴ CpG islands, regions with enriched CpG dinucleotides, are usually located at the 5' end of the genes.⁴⁹ Methylation of DNA at the gene promoter inhibits gene transcription, by limiting the accessibility of the transcription machinery or by engaging proteins, such as HDACs, that repress gene expression.⁵⁵ De novo DNA methyltransferases (DNMT), DNMT3A and DNMT3B, are crucial for establishing and maintaining the global methylation pattern found in the genome, showing no preference for unmethylated or hemimethylated DNA.^{56,57} DNMT1 functions to maintain the methylation status during cell replication and shows preference to hemimethylated DNA.⁵⁶

A study reported that hypermethylation of PRX II promoter was observed in A549 lung adenocarcinoma cells, where PRX II was observed to be frequently down-regulated.³⁶ Interestingly, demethylation was observed in the same study in A549/GR cells which highly expressed PRX II, demonstrating that methylation status correlates to gene activity. Moreover, it also suggests that PRX II could

play a role in gefitinib resistance. In oligodendroglial tumors, methylation analysis revealed that PRX I-associated CpG island was frequently hypermethylated in 1p/19q-deleted primary tumors and expressed reduced PRX I mRNA transcripts.⁴¹ In addition, DNA hypermethylation and H3 histone hypoacetylation, also correlated with repressed tumor suppressor PRX II expression in acute myeloid leukemia (AML) and classic Hodgkin lymphoma.^{58,59}

Histone modification

The post-transcriptional modifications of histones occur predominantly on the amino-terminal and carboxyl-terminal of the histone tails.⁶⁰ These modifications include ribosylation, phosphorylation of serines and threonines, acetylation of lysines and arginines, ubiquitylation, and sumoylation of lysines.^{49,60,61} Collectively, these modifications make up the "histone code" that dictates chromatin activity, which can then regulate fundamental cellular processes⁴⁹ and also ensure global cell type-specific gene expression.⁶² For instance, acetylation of lysine residues correlates with transcriptionally active genes,⁶³ while methylation of specific lysine can lead to either transcriptional gene activation or repression. Methylation of H3K4, H3K36, and H3K79 is known to activate genes, whereas H3K9, H3K27, and H4K20 methylation have been implicated in transcriptional repression.⁶⁴

Among the 18 HDACs known, HDAC6 was found to specifically target PRX I and II, indicating that epigenetics could be an important factor in PRXs regulation.⁶⁵ HDAC6 has been observed to decrease the H₂O₂ reducing capacity of PRX I and II, which render them more prone to hyperoxidation. It was demonstrated that downregulation of PRX IV in acute promyelocytic leukemia was associated with increased lysine residue methylation of H3K27me3 and H3K4me3, at the promoter of PRX IV but not CpG hypermethylation.⁶⁶ It would seem that the activity of PRXs could be indirectly controlled by epigenetic mechanisms. As previously mentioned, hyperoxidation of PRXs can be reversed by sestrin 2, an enzyme that is epigenetically regulated, whereby histone acetylation by HDACi trichostatin A (TSA) treatment can enhance sestrin 2 transcription and thus contribute to increased PRX activity.¹⁰

miRNAs

miRNAs belong to a class of small non-coding RNAs (~22 nucleotides) that regulate expression of genes implicated in crucial cellular processes, such as proliferation, apoptosis, development, and differentiation.^{67–69} These miRNAs cleave target mRNAs or inhibit protein translation based on the complementarity to their targets. High complementarity between miRNA and its target mRNA results in mRNA cleavage via a RNA interference (RNAi) mechanism, while insufficient complementarity results in translational repression. Thus, the targeted gene will then be effectively repressed.

The association between cancer and miRNAs was first made, when it was discovered that the majority of chronic lymphocytic leukemia patients have deleted or down-regulated miR-15a and miR-16-1.⁷⁰ Since then,

miRNA profiling using miRNA microarray has shown that certain miRNA families are frequently deregulated in cancers. Based on current understanding of miRNAs, the most plausible mechanism by which miRNAs influence carcinogenesis is their ability to silence key tumor suppressors or regulators of oncogenes, thereby promoting cancer onset and progression.

A handful of miRNAs that target PRXs implicated in cancer progression are briefly described below.

miR-510. In earlier reports, miR-510 has been implicated in irritable bowel syndrome, breast cancer, epithelial ovarian cancer, and renal cell carcinoma.^{71–75} In breast cancer cells, miR-510 overexpression led to a more aggressive phenotype observed by targeting PRX I. Moreover, miR-510 overexpressing cells were more sensitive to H₂O₂ treatment. It would seem that miR-510 has the propensity of a novel "onco-miR," promoting carcinogenesis.⁷³

miR-122a. Liver-specific miR-122a has been reported to directly target PRX II in HCC.⁷⁶ Overexpression of miR-122a has been demonstrated to inhibit cell growth by enhancing apoptosis in HCC. In addition, miR-122a has also been shown to target cyclin G1 in HCC, suggesting that miR-122a could be possibly involved in the pathogenesis of liver cancer.

miR-23b and miR-383. miR-23b has been shown to alter PRX III expression levels in prostate cancer and AML.^{77,78} Immunohistochemical analysis showed reduced expression of miR-23b associated with metastatic prostate cancer tissue. Interestingly, miRNA profiling revealed that miR-23b clusters are frequently downregulated in prostate cancer, which may account for the frequent PRX III up-regulation observed in prostate cancer.⁷⁹ In medulloblastoma, enhanced miR-383 expression was observed to decrease cell growth, increase the number of cells in the sub-G1 phase of the cell cycle, and alter expression of proteins related to apoptosis.⁸⁰ Furthermore, depletion of PRX III using siRNA resulted in similar effects, indicating that the phenotypic changes observed were due to silencing of PRX III by miR-383.

miR-214. An *in vivo* study on radiation-induced skin injury showed that PRX VI expression was increased by the miR-124 inhibitor, leading to beneficial effects such as decreased radiation-induced ROS, mitochondrial and skin damage in rats.⁸¹

A summary of the miRNAs that regulate the different PRXs and their functional effects is shown in Table 1.

Future perspective. There is increasing evidence that PRXs play important roles in different aspects of carcinogenesis. Furthermore, studies have demonstrated that de-regulated PRXs levels and associated disease phenotypes can be restored to the healthy state with the use of miRNAs and epigenetic regulators that target PRXs. Therefore, PRXs and its epigenetic regulators could be exploited for therapeutic development. For example, HDAC6 was discovered to target

Table 1 Biological effects of miRNAs associated with PRX

miRNA	Targeted PRX	Biological effects	References
miR-510	PRX I in breast cancer	Overexpression of miR-510 <ul style="list-style-type: none"> Increased proliferation, migration, invasion, colony formation in vitro, and tumor growth in vivo Higher cell death when treated with H₂O₂ indicating compromised antioxidant system 	Guo et al. ⁷³
miR-122a	PRX II in hepatocellular carcinoma	Overexpression of miR-122a inhibited cell growth by induction of apoptosis	Diao et al. ⁷⁶
miR-23b	PRX III in prostate cancer	Immunohistochemistry indicate the association of expression level of miR-23b and PRX3 with severity of tumor malignancy	He et al. ⁷⁷
miR-383	PRX III in medulloblastoma	<ul style="list-style-type: none"> Overexpression of miR-383 suppressed cell growth, accumulation of cells at sub-G1 phase, and alterations of apoptosis-related proteins siRNA-mediated depletion of PRX3 gives rise to the same phenotype as miR-383 overexpression 	Li et al. ⁸⁰
miR-214	PRX VI in rat skin tissues after irradiation	miR-214 inhibitors <ul style="list-style-type: none"> Protect skin cells against radiation-induced damage by decreasing ROS Increased mitochondrial integrity after irradiation 	Zhang et al. ⁸¹

PRX I promoter and impair its reducing capability. FK228, a HDAC inhibitor, has been observed to repress tumor growth and induce apoptosis by accumulating acetylated H3 and H4 in the PRX I promoter, thereby inducing activation of PRX I.⁸² This has led to approval by FDA for the use of FK228, also known as Romidepsin (Istodax®) in 2009, for the treatment of cutaneous T-cell lymphoma.⁸³ Similarly, as miRNAs have been reported to regulate PRXs in cancers, they could also be targets for a potential cancer therapy. The systemic delivery of RNA oligonucleotides such as miRNAs can regulate gene expression and change the phenotype. For example, MRX34, a miR-34a mimic which became the first miRNA to enter clinical trials, is known to elicit pro-apoptotic effects and abolish growth in liver cancer and diffuse large B-cell lymphoma.^{84,85}

While PRXs and its epigenetic regulators could possibly serve as promising therapeutic targets, the use of epigenetic regulators such as HDACi is not without limitations. This is because the effects are usually genome wide, leading to undesirable upregulation of certain genes, such as pro-metastatic genes and also genes associated with drug resistance.⁸⁶ Therefore, refinement of epigenetic therapy is crucial to improve the efficacy of the treatment.⁸⁷ This can be done by epigenetic editing which involves the fusion of an enzyme to a specific DNA binding domain, enabling it to selectively target a specific gene and reshaping the epigenetic landscape.⁸⁶ For instance, fusion of DNMT3a with zinc finger transcription factor targeting the promoters of Maspin and SOX2, is capable of specific and efficient downregulation of these genes with stable phenotypic reprogramming of cancer cells.⁸⁸

In sum, PRXs serve as attractive molecular targets for effective cancer therapeutics.

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created the figures. BHB supervised the writing, amended and edited the manuscript. All authors read and approved the final manuscript.

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