Peroxiredoxin 6 (Prdx6) supports NADPH oxidase 1 (Nox1)-based reactive oxygen species generation and cell migration: collaboration of oxidant generating and scavenging systems


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Abstract

Nox1 is an abundant source of reactive oxygen species (ROS) in colon epithelium recently shown to function in wound healing and epithelial homeostasis. We identified Peroxiredoxin 6 (Prdx6) as a novel binding partner of Nox1 activator (Nox1) in yeast two-hybrid screening experiments using the Nox1 SH3 domain as bait. Prdx6 is a unique member of Prdx family that exhibits both glutathione peroxidase and phospholipase A2 activities. We confirmed this interaction in cells overexpressing both proteins, showing Prdx6 binds to and stabilizes wild-type Nox1, but not the SH3 domain mutant form, Nox1 W436R. We demonstrated in several cell models that Prdx6 knockdown suppressed Nox1 activity, whereas enhanced Prdx6 expression supports higher Nox1-based ROS release. Prdx6-dependent enhanced ROS production by Nox1 depends on both the peroxidase and phospholipase A2 activities of Prdx6, since peroxidase and lipase-deficient mutant forms failed to bind to or stabilize Nox1 components or support Nox1-mediated ROS generation. Furthermore, wild-type Prdx6, but not lipase or peroxidase mutant forms, supports Nox1-mediated cell migration in the HCT-116 colon epithelial cell model of wound closure. These findings highlight a novel pathway in which an antioxidant enzyme positively regulates an oxidant-generating system to support cell migration and wound healing.

Introduction

Reactive oxygen species (ROS) are now regarded as important signaling molecules in biological systems and have diverse roles in health and disease. Along with mitochondria, a family of NADPH oxidases (Nox) has been identified as a major source of ROS in many cell types. These enzymes are membrane-integrated protein complexes that utilize molecular oxygen and NADPH to generate superoxide anion (O2•−). Among the Nox enzymes, the Nox1/Nox2 (a.k.a., gp91phox) heterodimer is the best characterized. Nox family members are identified, is most abundant in colon epithelial cells recently shown to function in wound healing and epithelial homeostasis. Nox1 is an abundant source of ROS in colon epithelium recently shown to function in wound healing and epithelial homeostasis. We identified Peroxiredoxin 6 (Prdx6) as a novel binding partner of Nox1 activator (Nox1) in yeast two-hybrid screening experiments using the Nox1 SH3 domain as bait. Prdx6 is a unique member of Prdx family that exhibits both glutathione peroxidase and phospholipase A2 activities. We confirmed this interaction in cells overexpressing both proteins, showing Prdx6 binds to and stabilizes wild-type Nox1, but not the SH3 domain mutant form, Nox1 W436R. We demonstrated in several cell models that Prdx6 knockdown suppressed Nox1 activity, whereas enhanced Prdx6 expression supports higher Nox1-based ROS release. Prdx6-dependent enhanced ROS production by Nox1 depends on both the peroxidase and phospholipase A2 activities of Prdx6, since peroxidase and lipase-deficient mutant forms failed to bind to or stabilize Nox1 components or support Nox1-mediated ROS generation. Furthermore, wild-type Prdx6, but not lipase or peroxidase mutant forms, supports Nox1-mediated cell migration in the HCT-116 colon epithelial cell model of wound closure. These findings highlight a novel pathway in which an antioxidant enzyme positively regulates an oxidant-generating system to support cell migration and wound healing.

Materials & Methods

Results

I. Wild Prdx6 associates with the SH3 domain of Nox1 and stabilizes its expression

Two enzymes in one protein Prdx6

Figure 1. Two enzymes in one protein Prdx6

Prdx6 supports Nox1 NADPH oxidase activity

VI. Stability of the Nox1-supportive components is reduced by expression of a low activity mutant of Nox1

Figure 6. (A) Western blot demonstrating the expression of WT and low activity mutant Nox1 plasmids (0.2 ug each) were cotransfected with Nox1 components along with vector (mock), Prdx6 (0.8 ug each). After 48 hrs post-transfection, cells were treated with TNF and analyzed by Western blot analysis. (B). Expression of a low activity mutant of Nox1 reduces the formation of oxidant generator as determined using Diogenes luminescence assay. (C). Expression of a low activity mutant of Nox1 reduces the formation of oxidant generator as determined using Diogenes luminescence assay. (D). Endogenous Nox1 and Prdx6 were analyzed in cell lines reconstituted with WT and low activity mutant Nox1 defined by Western blotting (right).

Prdx6 modulates Nox1-derived ROS generation and migration of HCT-116 colon epithelial cells

Figure 7. (A) Representative cell migration images of HCT-116 colon epithelial cell lines expressing Prdx6 and Nox1 wild type (Nox1 WT) and low activity mutant (Nox1 S32A). Prdx6 and Nox1 WT plasmids were coexpressed (Prdx6/Nox1 WT). Prdx6 and Nox1 S32A plasmids were coexpressed (Prdx6/Nox1 S32A). Cells were seeded in Transwell filter inserts and allowed to migrate in the presence of NAPD (48 hrs). Photographs were acquired under oil immersion at a magnification of 20X. Right panel shows corresponding Western blot analysis of expressed proteins. All data represent mean ± S.D. (n=3).

Summary

We identified Peroxiredoxin 6 (Prdx6) as a novel positive regulator of the Nox1-based reactive oxygen generating enzyme complex. Earlier work showed that Prdx6 also regulates the Nox2 based oxidase; the closest relative of Nox1.

Prdx6 was initially identified as an interacting partner of the SH3 domain of Nox1. Interaction of the full-length proteins was confirmed in several transfected cell models. We confirmed the interaction of full-length Prdx6 and Nox1 proteins in various cell lines. Overexpressed Prdx6 stimulated cell migration in a wound assay, whereas Nox1 knockdown cells were rescued by Prdx6 expression. These results provide evidence of Prdx6 as a modulator of Nox1 activity. Prdx6 interacts with Nox1 at the SH3 domain, increasing its stability and function. This interaction allows Prdx6 to regulate Nox1-mediated ROS production and cell migration. Combination of Prdx6 and Nox1 activity promotes cell migration, whereas inhibition of either protein reduces cell motility. In conclusion, Prdx6 modulates Nox1-mediated ROS generation and migration of HCT-116 colon epithelial cells.