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SOD1 deficiency induces the systemic hyperoxidation of peroxiredoxin in the mouse

Takujiro Homma [a], Satoshi Okano [b], Jaeyong Lee [a], Junitsu Ito [a], Noriyuki Otsuki [a], Toshihiro Kurahashi [a], Eun Sil Kang [a], Osamu Nakajima [b], Junichi Fujii [a, *]

[a] Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, Yamagata University, 2-2-2 Iidanishi, Yamagata 990-9585, Japan
[b] Research Laboratory for Molecular Genetics, Yamagata University School of Medicine, Yamagata University, 2-2-2 Iidanishi, Yamagata 990-9585, Japan

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ABSTRACT

A deficiency of superoxide dismutase 1 (SOD1) or peroxiredoxin (Prx) 2 causes anemia in mice due to elevated oxidative stress. In the current study, we investigated whether intrinsic oxidative stress caused by a SOD1 deficiency affected the redox status of Prx2 and other isoforms in red blood cells (RBCs) and several organs of mice. We observed a marked elevation in hyperoxidized Prx2 levels in RBCs from SOD1-deficient mice. Hyperoxidized Prx2 reportedly undergoes a rhythmic change in isolated RBCs under culture conditions. We confirmed such changes in RBCs from wild-type mice but observed no evident changes in SOD1-deficient RBCs. In addition, an elevation in hyperoxidized Prxs, notably Prx2 and Prx3, was observed in several organs from SOD1-deficient mice. However, a SOD1 deficiency had no impact on the wheel-running activity of the mice. Thus, although the redox status of some Prxs is systemically shifted to a more oxidized state as the result of a SOD1 deficiency, which is associated with anemia and some diseases, a redox imbalance appears to have no detectable effect on the circadian activity of mice.

1. Introduction

Oxidative stress emerges under conditions involving either an elevated production of reactive oxygen species (ROS) or decreased antioxidative/redox capacity. We previously reported that ROS levels are elevated in RBCs and an anemic phenotype in SOD1-deficient mice [1]. Anemia induced by the absence of SOD1 appears to be caused by accelerated hemolysis in the blood plasma and the phagocytotic removal of RBCs by liver macrophages, namely Kupffer cells [2]. While SOD1 is the sole enzyme that scavenges superoxide in RBCs, several enzymes are involved in the detoxification of peroxides and include glutathione peroxidase 1 (GPX1), catalase (CAT), and peroxiredoxin (Prx).

Among the six isozymes of Prx in mammals, Prx2 is present predominantly in RBCs, which are the third most abundant protein in RBCs [3,4]. Prx2 is postulated to play a greater role than GPX1 in removing endogenous hydrogen peroxide [5]. While a deficiency of neither GPX1 nor CAT results in the development of a hematological abnormality in mice [6,7], both Prx1-and Prx2-knockout mice develop anemia [8,9]. Thus, in addition to SOD1, Prx2 appears to play an essential role in protecting RBC from oxidative stress triggered by peroxides [5].

The reaction of the sulfhydryl group of cysteine (Cys-SH) with hydrogen peroxide forms cysteine-sulfenic acid (Cys-SOH) that is either reduced back to Cys-SH, subjected to further oxidation to sulfinic (Cys-SO_2H) and sulfonic acid (Cys-SO_3H), or reacts with another sulfhydryl to form a disulfide [10,11]. Prx2 consists of a homodimer with a head-to-tail antiparallel orientation [12] and subunits transiently form a disulfide during catalysis [13,14]. Prx also undergoes hyperoxidation in the catalytic conversion of Cys-SH to Cys-SOH by the reaction with hydrogen peroxide and is further oxidized to Cys-SO_2H and consequently Cys-SO_3H [15–18]. Although Cys-SO_2H can be converted back to Cys-SH by the action of sulfiredoxin in an ATP-dependent manner [19], sulfiredoxin is much less abundant than Prx2 in RBC, which leads to the accumulation of hyperoxidized Prx2. Recent studies have shown that the levels of hyperoxidized Prx2 in human and mouse RBCs

Abbreviations: RBC, red blood cell; ROS, reactive oxygen species; SOD, superoxide dismutase; Prx, peroxiredoxin; GPX, glutathione peroxidase; CAT, catalase; CAII, carbonic anhydrase II; Cys-SOH, cysteine sulfenic acid; Cys-SO_2H, cysteine sulfinic acid; Cys-SO_3H, cysteine sulfonic acid.

* Corresponding author.
E-mail address: jfujii@med.id.yamagata-u.ac.jp (J. Fujii).

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undergo rhythmic changes similar to a circadian rhythm, even under culture conditions [20,21]. However, the issue of whether or not the hyperoxidized form is merely a marker reflecting the redox state of RBCs or actually plays a role in circadian rhythm in the body is not known with certainty [22]. Thus, the physiological significance of the hyperoxidation of Prx2 and its rhythmic change remain to be clarified.

The findings reported herein indicate that high levels of hyperoxidized Prx2 at the catalytic Cys-SH were sustained in SOD1-deficient RBCs and no rhythmic change was observed under culture conditions. We then examined whether or not the SOD1 deficiency caused a systemic redox shift in Prxs and affected the circadian activity of the mice.

2. Materials and methods

2.1. Antibodies

Anti-Peroxiredoxin-$SO_{2/3}$ (Abcam, ab16830), anti-β-actin (Santa Cruz Biotechnology, sc-69879), anti-CAII (Santa Cruz Biotechnology, sc-17244), anti-Prx2 (AbFrontier, LF-PA0091), and anti-GAPDH (Santa Cruz Biotechnology, sc-25778) were purchased from the indicated vendors. Horseradish peroxidase-conjugated anti-goat (sc-2020), anti-mouse (sc-2005), and anti-rabbit (sc-2004) IgG antibodies were purchased from Santa Cruz Biotechnology. The anti-human SOD1 antibody used in this study has been described elsewhere [1].

2.2. Mice

C57BL/6 *SOD1*$^{+/-}$ mice, originally established by Ref. [23], were purchased through Jackson Laboratories (Bar Harbor, ME, USA) and bred at our institute, giving rise to *SOD1*$^{+/+}$ (wild-type: WT) and *SOD1*$^{-/-}$ (SOD1-deficient) littermates. A genotypic analysis of the mice was performed using PCR with specific primers, as previously described [1]. Transgenic mice expressing human SOD1 under the GATA1 promoter have been described previously [24]. The mice were backcrossed to C57BL/6 mice more than eight times before being used in this study. The animal room was maintained under specific pathogen-free conditions at a constant temperature of 20–22 °C with a 12 h alternating light–dark cycle. Animal experiments were performed in accordance with the Declaration of Helsinki under the protocol approved by the Animal Research Committee at Yamagata University.

2.3. Culture of RBCs

Culturing of mouse RBCs was performed as previously described by Cho and co-workers [21] with minor modifications. Briefly, blood (0.3 mL) was collected from wild-type and SOD1-deficient mice through the abdominal vein, transferred to a 1.5 mL tube containing 100 μL of an acid citrate dextrose solution (22.0 g/L sodium citrate, 7.3 g/L anhydrous citric acid, and 24.5 g/L glucose), and centrifuged at 1500 × g for 10 min at room temperature. The RBC pellet was washed three times with PBS, and suspended in DMEM (Wako, 044-29765) supplemented with 110 mg/L sodium pyruvate, 0.1% BSA, 100 U/mL penicillin, and 100 mg/mL streptomycin, and, after being dispensed into 1.5 mL tubes at a density of 1.5×10^7 cells/50 μL; they were maintained at 37 °C in the dark under an atmosphere of 5% CO_2 and 20% O_2 in air. Tubes were removed from the incubator every 4 h after isolation for the addition of 2 × SDS sample buffer, then incubated at 95 °C for 10 min, and stored at −80 °C until needed.

2.4. Preparation of mouse organs

The isolation and preparation of mouse organs has been described previously [25]. Briefly, organs (liver, kidney, lung, spleen) were excised from mice under anesthesia and quickly frozen in liquid nitrogen, and stored in a deep freezer until used. The samples were homogenized with a glass-Teflon homogenizer in lysis buffer (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing 100 mM N-ethylmaleimide (Wako, 058-02061) and protease inhibitor cocktail (Sigma, P8340). After centrifugation, the protein concentration was determined using Pierce® BCA™ Protein Assay Kit (Pierce).

2.5. Immunoblotting

The proteins were separated on 12% or 15% SDS–polyacrylamide gels and blotted onto polyvinylidene difluoride (PVDF) membranes (GE Healthcare). The blots were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), and were then incubated overnight with the primary antibodies diluted in TBST containing 1% skim milk. After three washes in TBST, the blots were incubated with a horseradish peroxidase-conjugated second antibody. After washing, the bands were detected using Immobilon western chemiluminescent HRP substrate (Millipore) on an image analyzer (ImageQuant LAS500, GE Healthcare).

2.6. Measuring wheel-running activity of mice

Three male wild-type and SOD1-deficient mice at 10 weeks of age were subjected to the wheel-running as described [26]. We examined rhythmic change of physical activity by monitoring wheel-running activity under constant darkness (DD), following 12 h light/12 h dark (LD) cycles.

2.7. Statistical analyses

Statistical analyses of the data were performed using GraphPad Prism 4 software. P-values were calculated using the Student's *t*-test.

3. Results

3.1. Elevated hyperoxidized Prx2 in SOD1-deficient RBCs

To examine whether a SOD1 deficiency affected the oxidation state of Prx2, we performed immunoblotting and detected hyperoxidized Prx (Prx-SO_2/SO_3) as well as total Prx2. While the content of hyperoxidized Prx was quite low in freshly isolated wild-type RBCs, the levels were originally high in SOD1-deficient RBCs (Fig. 1A). RBCs contain both Prx1 and Prx2 that cannot be separated from each other by SDS-PAGE due to their similar molecular sizes. The epitope sequences to anti- Prx-SO_2/SO_3 antibodies are the same between Prx1 and Prx2, so that the reactive bands might contain both isoforms. However, it has been reported that Prx1 is not hyperoxidized as demonstrated by using Prx2-deficient mouse RBCs [21]. Therefore, the hyperoxidized Prx in the wild-type and SOD1-deficient RBCs are likely to be Prx2. The total protein levels of Prx2 were not significantly changed in SOD1-deficient RBCs compared with wild-type RBCs (Fig. 1B). In the RBCs of wild-type mice, ~0.9% of the Prx2 is reported to be hyperoxidized [21]. The relative amount of hyperoxidized Prx2 was about seven-times larger in SOD1-deficient RBCs than in wild-type RBCs (Fig. 1A). These results indicate that endogenous oxidative stress due to a SOD1 deficiency exacerbated the hyperoxidation of Prx2 but did not exert a large influence over the total protein levels of Prx2.

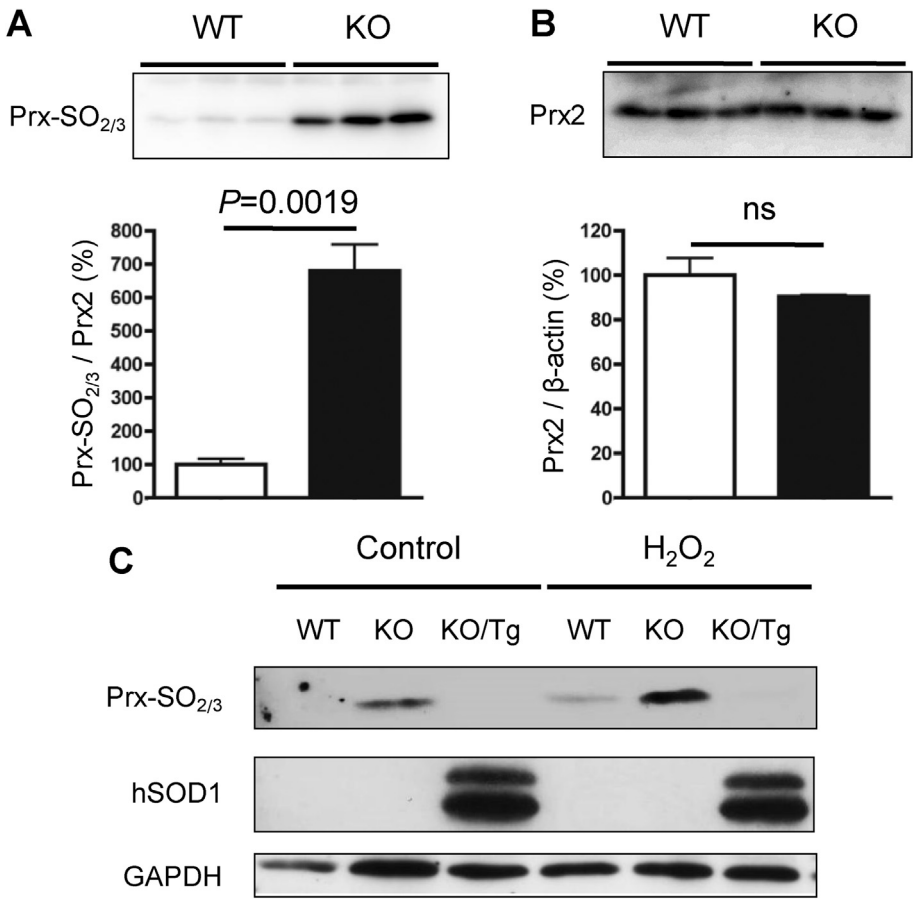

Fig. 1. Levels of hyperoxidized Prx in wild-type and SOD1-deficient RBCs. (A, B) Representative immunoblots of wild-type (WT) and SOD1-deficient (KO) RBCs probed with antibodies against Prx-$SO_{2/3}$ (A) and total Prx2 (B) are shown (n = 3 for each group). Bottom panels depict the quantification of each protein normalized to the corresponding β-actin and Prx2, respectively. Values are the mean ± SD of mice. ns, not significant. (C) Wild-type (WT), SOD1-deficient (KO), and SOD1-deficient/human SOD1-Tg (KO/Tg) RBCs were exposed to 1 mM hydrogen peroxide (H_2O_2) for 30 min, and subjected to immunoblotting. The representative immunoblots probed with antibodies against Prx-$SO_{2/3}$, hSOD1, and GAPDH are shown.

We previously established SOD1-deficient mice that lack the SOD1 protein in the whole body but express human SOD1 in RBC by crossing a strain of transgenic mice overexpressing human SOD1 only in erythroid cells (Tg) [24] with a SOD1-deficient mouse. Here we examined whether the transgenic overexpression of human SOD1 suppressed the hyperoxidation of Prx2 in RBC of SOD1-deficient mice (Fig. 1C). We found that the transgenic expression of human SOD1 completely suppressed the Prx2 hyperoxidation in RBCs exposed to 1 mM hydrogen peroxide. Because the expression of the human SOD1 gene is driven under the GATA1 promoter in this Tg mouse [24], this result indicates that the hyperoxidation of Prx2 occurred after being produced in erythroid cells.

3.2. Impaired redox oscillations of hyperoxidized Prx2 in SOD1-deficient RBCs

Rhythmic changes in hyperoxidized Prx2 levels according to circadian rhythm have been reported in human and mouse RBCs in culture [20,21]. It would be intriguing to know if the hyperoxidized Prx2 also underwent rhythmic changes in cultured RBCs under elevated oxidative stress produced by a SOD1 deficiency. We examined the changes in the levels of hyperoxidized Prx2 in cultured RBCs obtained from wild-type and SOD1-deficient mice at 4-h intervals for 24 h (Fig. 2A). The levels of hyperoxidized Prx2 in RBCs from wild-type mice changed rhythmically, consistent with previous reports [20,21]. In contrast, the Prx2 in SOD1-deficient RBCs was markedly hyperoxidized at the beginning of the culture and its oxidation level remained essentially unchanged during the incubation period (Fig. 2B). The levels of carbonic anhydrase II (CAII), which is a non-relevant and the second most abundant protein in RBCs, were not significantly changed during the incubation in both RBCs (Fig. 2C and D).

3.3. Elevated hyperoxidized Prxs in SOD1-deficient mouse organs

In order to examine the systemic redox state of Prxs, we performed immunoblotting and detected hyperoxidized Prxs in several mouse organs (liver, kidney, spleen, lung). Because most organs express both Prx1 and Prx2, the sizes of which are similar, and we cannot say with certainty which was the hyperoxidized isoform, the hyperoxidized proteins corresponding to a molecular weight of 22 kDa were labeled Prx1/2 here. The hyperoxidized form with a weight of 24 kDa was identical to a mitochondrial isoform of Prx3. While the contents of the hyperoxidized Prxs were quite low in freshly isolated wild-type mouse organs, the levels were markedly elevated in all organs from SOD1-deficient mice (Fig. 3). It is noteworthy that, in addition to Prx1/2, Prx3 in the liver and kidney was also hyperoxidized to a considerable extent. These results indicate that a SOD1 deficiency disturbs the Prx redox system systemically.

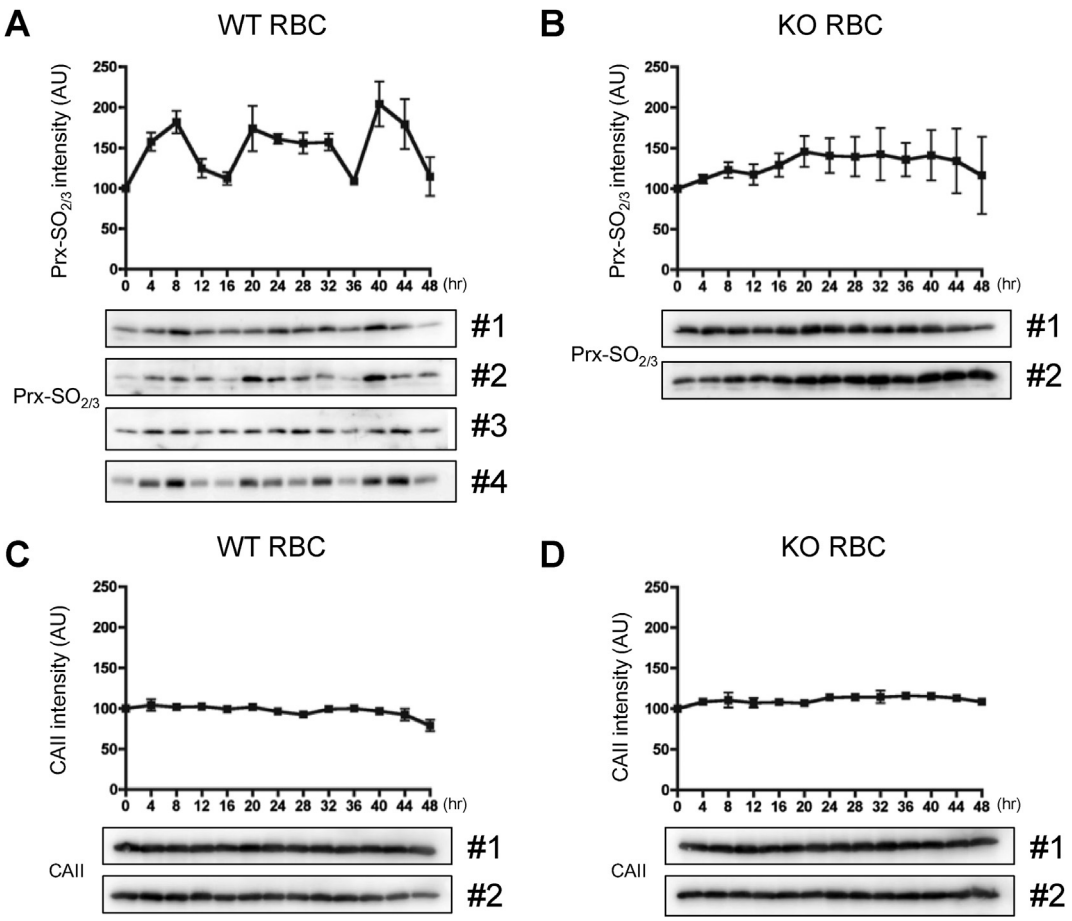

Fig. 2. The oscillation of hyperoxidized Prx2 in wild-type and SOD1-deficient RBCs. Wild-type (WT) and SOD1-deficient (KO) RBCs from each type of mice were incubated in DMEM-based media at 37 °C in the dark in a CO_2 incubator. Proteins were extracted from each RBCs every 4 h after isolation, and subjected to immunoblotting. The representative immunoblots probed with antibodies against Prx-$SO_{2/3}$ (A, B) and CAII (C, D) are shown.

3.4. No correlation between oxidative oscillation of Prx and the circadian pattern of physical activity was found in mice

The issue of whether the change in the oxidation state of Prx2 is actually the cause of the circadian rhythm or simply a result is debatable [22]. To address this, we next asked if the systemic hyperoxidation of Prxs in organs might be correlated with the wheel-running activity of SOD1-deficient mice. SOD1-deficient mice were entrained to LD12:12 following the same procedure as the wild-type controls. No significant difference was observed between wild-type and SOD1-deficient mice in the activity pattern in LD12:12 conditions (Fig. 4A). After returning to LD from DD, no obvious abnormality in entrainment behavior was observed in the SOD1-deficient mice again. Under DD, the SOD1-deficient mice displayed robust circadian rhythms in wheel-running activity. The SOD1-deficient mice showed the same free-running periods as the wild-type mice (Fig. 4B). Thus there appeared to be no significant correlation between the hyperoxidation of Prxs and wheel-running activity of the mice.

4. Discussion

While the hyperoxidation of Prxs typically occurs under conditions where peroxides are overproduced, such as inflammation and ischemia reperfusion, the physiological significance of this is largely unknown [22]. In the current study, we show (for the first time) that the catalytic sulfhydryl of the Cys residue of Prx2 was markedly hyperoxidized in SOD1-deficient RBCs compared to wild-type RBCs (Fig. 1B). The transgenic expression of human SOD1 in RBCs of SOD1-deficient mice suppressed the Prx2 hyperoxidation to the levels of wild-type RBCs (Fig. 1C), which fulfills the absence of SOD1 as a sole determinant of the oxidation state of Prx2 in SOD1-deficient RBCs. Because human SOD1 produced in RBCs ameliorates the anemia in SOD1-deficient mice [24] and at the same time suppressed Prx2 hyperoxidatoin, the inactivation of Prx2 as the result of hyperoxidation might be involved in the onset of the hemolytic anemia caused by a SOD1 deficiency. We also found an elevated level of hyperoxidation of Prx1/2 in all organs examined and Prx3 in certain organs in SOD1-deficient mice (Fig. 3). Hydrogen peroxide is a common substrate for the peroxidation reaction of Prxs and also a well-known cause for their hyperoxidation [15–18].

In a previous study, we showed that elevated oxidative stress in SOD1-deficient RBCs triggers the conversion of hemoglobin to methemoglobin and the oxidative modification of various molecules such as lipids, consequently leading to the development of anemia [1]. Superoxide produced from the autoxidation of hemoglobin is reportedly involved in the hyperoxidation of Prx2 in RBCs [21]. Hemoglobin is present at a concentration of 5 mM in RBC and the autoxidation of hemoglobin occurs at a rate of 2–3% in humans and 4% in the mouse a day and, hence, would be a major source for superoxide in RBCs [27,28]. Superoxide is spontaneously converted to hydrogen peroxide, and the conversion is accelerated by the catalytic action of SOD. In SOD1-

deficient RBCs, the levels of hydrogen peroxide in RBCs are predicted to be slightly less than those in wild-type RBCs [28]. Thus, it remains unclear how a SOD1 deficiency stimulates the hyperoxidation of Prxs.

Previous studies have reported the existence of rhythmic changes in hyperoxidized Prx2 levels in RBC in culture [20,21]. Cho et al. [21] also demonstrated that, in fact, hyperoxidized Prx2 is not converted back to the reduced form by the action of sulfiredoxin but, rather, undergoes proteolytic degradation by 20S proteasomes. While the hyperoxidation of Prx2 exhibited a rhythmic change in wild-type RBCs, hyperoxidized Prx2 levels remained high in SOD1-deficient RBCs and this level did not change in culture conditions (Fig. 2). Taken together, it is likely that proteosomal removal is impaired in SOD1-deficinet RBCs (submitted for publication), which results in the elevation in the hyperoxidized Prx2 under a SOD1 deficiency. Based on these data, we hypothesize that circadian activity is impaired by a SOD1 deficiency in mice and that the circadian oscillation of hyperoxidized Prxs levels plays a role in this process. It is noteworthy that no difference was observed in wheel-running activity between the two genetic groups of mice (Fig. 4). SOD1 expression is under differential regulation by the *period* gene [29], but a SOD1 deficiency did not appear to influence wheel-running activity. The possibility still remains that a circadian change caused by other physiological activities might be affected by a SOD1 deficiency.

In conclusion, we found that the levels of hyperoxidized Prx2 were markedly elevated in SOD1-deficient RBCs and the high levels were sustained without any evidence of a rhythmic change under cultural conditions. Since a deficiency of Prx2 also causes anemia, decreased Prx2 activity due to elevated hyperoxidation may also be involved in SOD1-deficient hemolytic anemia. Although the hyperoxidation of Prxs was also elevated systemically under a SOD1 deficiency, the redox imbalance may not have a substantial influence over the circadian activity of the mouse.

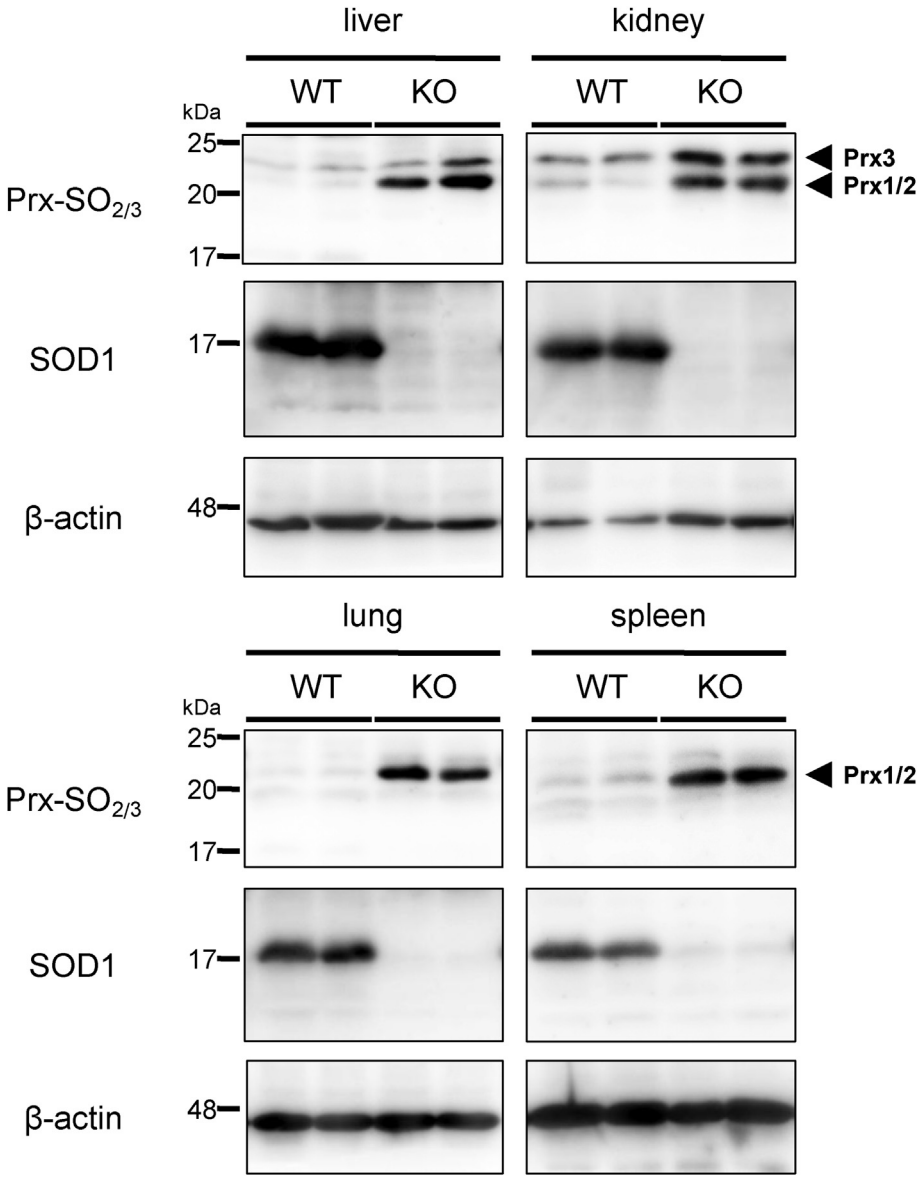

Fig. 3. Levels of hyperoxidized Prx (Prx-$SO_{2/3}$) in wild-type and SOD1-deficient mouse organs. The representative immunoblots of wild-type (WT) and SOD1-deficient (KO) mouse organs (liver, kidney, lung, spleen) probed with antibodies against Prx-$SO_{2/3}$, SOD1, and β-actin are shown (n = 2 for each group).

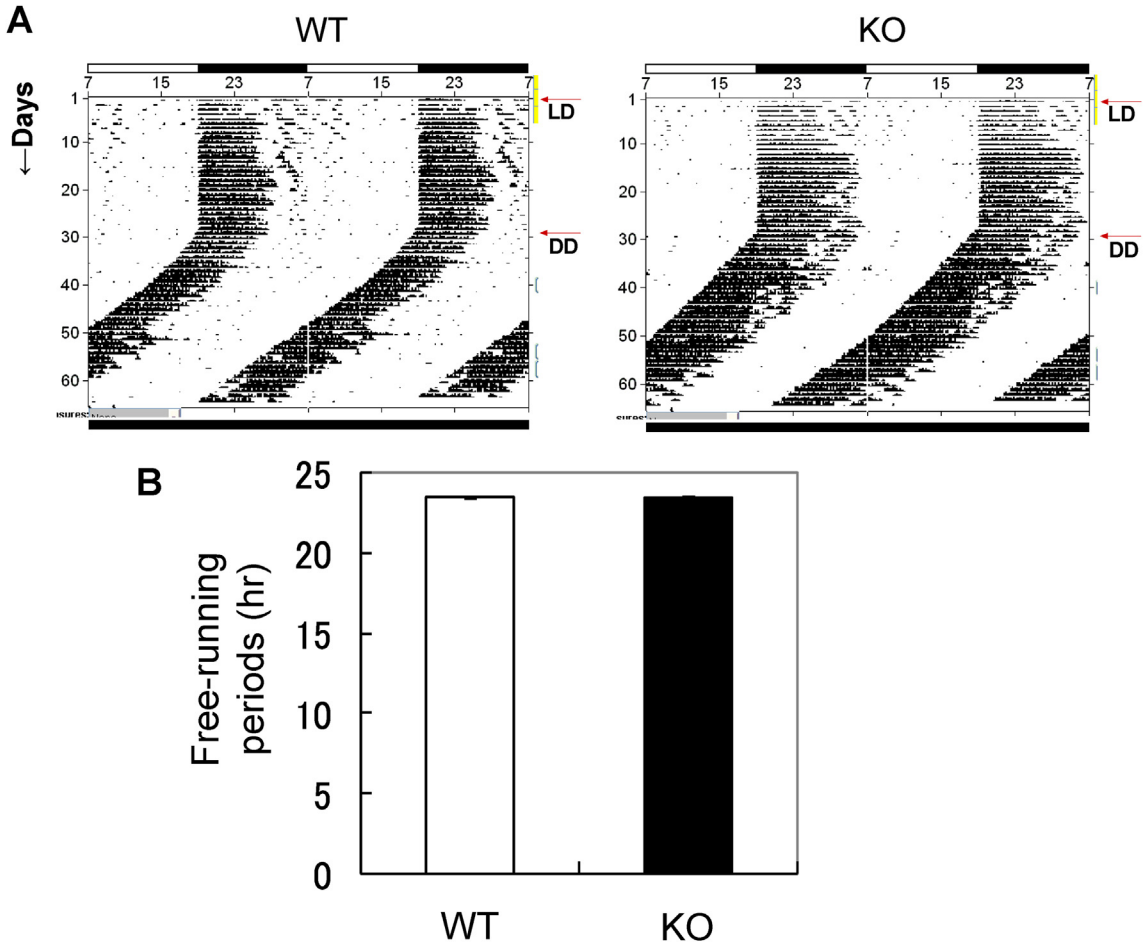

Fig. 4. Wheel-running activity in wild-type and SOD1-deficient mice. (A) The representative double-plotted actograms of wild-type (WT) and SOD1-deficient (KO) mice under LD, followed by DD conditions. (B) Average free-running period length of wild-type (WT) and SOD1-deficient (KO) mice. Bars: 100 μm. The graph represents the mean ± SE for three mice.

Conflicts of interest

We declare no conflict of interest between the authors or with any institution in relation to the contents of this article.

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References

[1] Y. Iuchi, F. Okada, K. Onuma, T. Onoda, H. Asao, M. Kobayashi, et al., Elevated oxidative stress in erythrocytes due to a SOD1 deficiency causes anaemia and triggers autoantibody production, Biochem. J. 402 (2007) 219–227, http://dx.doi.org/10.1042/BJ20061386.
[2] R.R. Starzyński, F. Canonne-Hergaux, A. Willemetz, M.A. Gralak, J. Woliński, A. Styś, et al., Haemolytic anaemia and alterations in hepatic iron metabolism in aged mice lacking Cu,Zn-superoxide dismutase, Biochem. J. 420 (2009) 383–390, http://dx.doi.org/10.1042/BJ20082137.
[3] R.B. Moore, M.V. Mankad, S.K. Shriver, V.N. Mankad, G.A. Plishker, Reconstitution of Ca2+- dependent K+ transport in erythrocyte membrane vesicles requires a cytoplasmic protein, J. Biol. Chem. 266 (1991) 18964–18968.
[4] F.M. Low, M.B. Hampton, C.C. Winterbourn, Peroxiredoxin 2 and peroxide metabolism in the erythrocyte, Antioxid. Redox Signal 10 (2008) 1621–1630, http://dx.doi.org/10.1089/ars.2008.2081.
[5] R.M. Johnson, Y.S. Ho, D.Y. Yu, F.A. Kuypers, Y. Ravindranath, G.W. Goyette, The effects of disruption of genes for peroxiredoxin-2, glutathione peroxidase-1, and catalase on erythrocyte oxidative metabolism, Free Radic. Biol. Med. 48 (2010) 519–525, http://dx.doi.org/10.1016/j.freeradbiomed.2009.11.021.
[6] R.M. Johnson, G. Goyette, Y. Ravindranath, Y.S. Ho, Red cells from glutathione peroxidase-1-deficient mice have nearly normal defenses against exogenous peroxides, Blood 96 (2000) 1985–1988.
[7] Y.S. Ho, Y. Xiong, W. Ma, A. Spector, D.S. Ho, Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury, J. Biol. Chem. 279 (2004) 32804–32812, http://dx.doi.org/10.1074/jbc.M404800200.
[8] C.A. Neumann, D.S. Krause, C.V. Carman, S. Das, D.P. Dubey, J.L. Abraham, et al., Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression, Nature 424 (2003) 561–565, http://dx.doi.org/10.1038/nature01819.
[9] T.-H. Lee, S.-U. Kim, S.-L. Yu, S.H. Kim, D.S. Park, H.-B. Moon, et al., Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice, Blood 101 (2003) 5033–5038, http://dx.doi.org/10.1182/blood-2002-08-2548.
[10] F.M. Low, M.B. Hampton, A.V. Peskin, C.C. Winterbourn, Peroxiredoxin 2 functions as a noncatalytic scavenger of low-level hydrogen peroxide in the erythrocyte, Blood 109 (2007) 2611–2617, http://dx.doi.org/10.1182/blood-2006-09-048728.
[11] J. Fujii, J.I. Ito, X. Zhang, T. Kurahashi, Unveiling the roles of the glutathione redox system in vivo by analyzing genetically modified mice, J. Clin. Biochem. Nutr. 49 (2011) 70–78, http://dx.doi.org/10.3164/jcbn.10-138SR.
[12] E. Schröder, J.A. Littlechild, A.A. Lebedev, N. Errington, A.A. Vagin, M.N. Isupov, Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes

at 1.7 Å resolution, Structure 8 (2000) 605–615, http://dx.doi.org/10.1016/S0969-2126(00)00147-7.
[13] J. Fujii, Y. Ikeda, Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein, Redox Rep. 7 (2002) 123–130, http://dx.doi.org/10.1179/135100002125000352.
[14] S.G. Rhee, H.A. Woo, Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H_2O_2, and protein chaperones, Antioxid. Redox Signal 15 (2011) 781–794, http://dx.doi.org/10.1089/ars.2010.3393.
[15] K.S. Yang, S.W. Kang, H.A. Woo, S.C. Hwang, H.Z. Chae, K. Kim, et al., Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid, J. Biol. Chem. 277 (2002) 38029–38036, http://dx.doi.org/10.1074/jbc.M206626200.
[16] J.C. Moon, Y.S. Hah, W.Y. Kim, B.G. Jung, H.H. Jang, J.R. Lee, et al., Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H 2O2-induced cell death, J. Biol. Chem. 280 (2005) 28775–28784, http://dx.doi.org/10.1074/jbc.M505362200.
[17] C.K. Lee, H.J. Kim, Y.R. Lee, H.H. So, H.J. Park, K.J. Won, et al., Analysis of peroxiredoxin decreasing oxidative stress in hypertensive aortic smooth muscle, Biochim. Biophys. Acta - Proteins Proteomics 1774 (2007) 848–855, http://dx.doi.org/10.1016/j.bbapap.2007.04.018.
[18] C.L. Jung, H.I. Choi, S.P. Yu, W.N. Hyung, A.W. Hyun, K.S. Kwon, et al., Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity, J. Biol. Chem. 283 (2008) 28873–28880, http://dx.doi.org/10.1074/jbc.M804087200.
[19] B. Biteau, J. Labarre, M.B. Toledano, ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin, Nature 425 (2003) 980–984, http://dx.doi.org/10.1038/nature02075.
[20] J.S. O'Neill, A.B. Reddy, Circadian clocks in human red blood cells, Nature 469 (2011) 498–503, http://dx.doi.org/10.1038/nature09702.
[21] C.-S. Cho, H.J. Yoon, J.Y. Kim, H.A. Woo, S.G. Rhee, Circadian rhythm of hyperoxidized peroxiredoxin II is determined by hemoglobin autoxidation and the 20S proteasome in red blood cells, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 1–6, http://dx.doi.org/10.1073/pnas.1401100111.
[22] G. Vogel, Cell biology. Telling time without turning on genes, Science 331 (2011) 391, http://dx.doi.org/10.1126/science.331.6016.391.
[23] M.M. Matzuk, L. Dionne, Q. Guo, T.R. Kumar, R.M. Lebovitz, Ovarian function in superoxide dismutase 1 and 2 knockout mice, Endocrinology 139 (1998) 4008–4011, http://dx.doi.org/10.1210/endo.139.9.6289.
[24] Y. Iuchi, F. Okada, R. Takamiya, N. Kibe, S. Tsunoda, O. Nakajima, et al., Rescue of anaemia and autoimmune responses in SOD1-deficient mice by transgenic expression of human SOD1 in erythrocytes, Biochem. J. 422 (2009) 313–320, http://dx.doi.org/10.1042/BJ20090176.
[25] J. Ito, N. Otsuki, X. Zhang, T. Konno, T. Kurahashi, M. Takahashi, et al., Ascorbic acid reverses the prolonged anesthetic action of pentobarbital in Akr1a-knockout mice, Life Sci. 95 (2014) 1–8, http://dx.doi.org/10.1016/j.lfs.2013.12.004.
[26] S. Okano, M. Akashi, K. Hayasaka, O. Nakajima, Unusual circadian locomotor activity and pathophysiology in mutant CRY1 transgenic mice, Neurosci. Lett. 451 (2009) 246–251, http://dx.doi.org/10.1016/j.neulet.2009.01.014.
[27] B.G.F. Howard FrankLin Bunn, Hemoglobin: Molecular, Genetic and Clinical Aspects, W.B. Saunders Co, 1986.
[28] R.M. Johnson, G. Goyette, Y. Ravindranath, Y.S. Ho, Hemoglobin autoxidation and regulation of endogenous H2O 2 levels in erythrocytes, Free Radic. Biol. Med. 39 (2005) 1407–1417, http://dx.doi.org/10.1016/j.freeradbiomed.2005.07.002.
[29] Y.S. Jang, M.H. Lee, S.H. Lee, K. Bae, Cu/Zn superoxide dismutase is differentially regulated in period gene-mutant mice, Biochem. Biophys. Res. Commun. 409 (2011) 22–27, http://dx.doi.org/10.1016/j.bbrc.2011.04.099.