Involvement of Prx3, a *Drosophila* ortholog of the thiol-dependent peroxidase PRDX3, in age-dependent oxidative stress resistance

Yasunari KAYASHIMA and Kimiko YAMAKAWA-KOBAYASHI

School of Food and Nutritional Sciences, Graduate School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, Japan

(Received 22 August 2012; and accepted 24 August 2012)

ABSTRACT

Peroxisredoxins (Prxs) are a family of multifunctional antioxidant thioredoxin-dependent peroxidases. We used *Drosophila melanogaster* to examine the function of Prx3, the *Drosophila* homolog of human PRDX3. The oxidative stress response in adult *Drosophila* is age-dependent. RNAi-induced *Prx3* knockdown in adult flies did not change their phenotype in normal conditions, but they had a shorter survival than the *Prx3*⁺ controls in the presence of H₂O₂. The expression levels of the *Prx3* were reduced by aging. These results suggest that Prx3 plays an important role in the oxidative stress response and is involved in the age-dependent competence of the oxidative stress response.

In multicellular organisms, the accumulation of reactive oxygen species (ROS) increases with age (10). This increase is considered to be attributed to its simple accumulation and to the impairment of the ROS scavenging capacity of the cell that is mainly due to dysfunction of the mitochondria, the major source of intracellular ROS (2, 12, 15). The incidence rates of cardiovascular diseases and neurodegenerative diseases also increase with age, indicating that ROS accumulation is a risk factor for age-related diseases (5, 14). Oxidative stress plays an important role in governing the life span of the fruit fly *Drosophila melanogaster* (4, 9). It is considered that responsiveness to oxidative stress declines with the number of days after eclosion. To determine whether the oxidative stress response alters with age in *Drosophila*, eclosed virgin male flies were isolated and kept in normal conditions for 2, 5 or 16 days, and then fed with food containing 0.5% H₂O₂. We

found that the survival of adult flies declined with the number of days after exposure to H₂O₂ (Fig. 1), suggesting that the oxidative stress response in adult *Drosophila* is impaired with age. This study demonstrated that the use of *Drosophila* enables the investigation of factors involved in aging-associated changes in response to oxidative stress.

Peroxisredoxins (Prxs) are a family of thiol peroxidases that decompose H₂O₂ and organic hydroperoxides (8). Human Prx3 (PRDX3) localizes to the mitochondrial matrix and displays strong reactivity to H₂O₂ (3). A mitochondrial dysfunction causes an abatement in ATP production, oxidative damage and the induction of apoptosis, all of which are involved in the pathogenesis of numerous disorders (11). Since all the members of PRDX family strongly scavenges H₂O₂ in a thioredoxin-dependent manner and PRDX3 is the only member of this family located in the mitochondria, PRDX3 is implicated in various age-related diseases that are considered to be caused either by accumulation of ROS or by insufficient ROS scavenging in mitochondria. However, actual involvement of PRDX3 in such age-related diseases is poorly understood. A homologous gene of PRDX family was found in *Drosophila*, and *Prx3*, the *Drosophila* ortholog of PRDX3, was identified on the basis of a genome-wide search (6).

Address correspondence to: Dr Yasunari Kayashima
School of Food and Nutritional Sciences, Graduate
School of Integrated Pharmaceutical and Nutritional
Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku,
Shizuoka 422-8526, Japan
Tel: +81-54-264-5226, Fax: +81-54-264-5220
E-mail: ykayashi@u-shizuoka-ken.ac.jp

Fig. 1 Competence of the oxidative stress response depends on aging in adult *Drosophila*. Wild-type (Oregon-R) adult males (20 per vial) were directly exposed to H₂O₂ by adding H₂O₂ to the 1.3% sucrose medium. 2 d, 5 d, and 16 d represent days after eclosion. Sample sizes (males): 2 d, n = 50; 5 d, n = 110; 16 d, n = 71.

Although the role of Prx3 against stressors was evaluated *in vitro* using cultured cells (7) and its expression during phagocytosis of *Candida albicans* has been reported (13), its *in vivo* role in the fly is not clear.

To investigate the involvement of Prx3 in the oxidative stress response *in vivo*, we used two transgenic fly lines (KD-1 and KD-2) in which the expression of the *Prx3* was specifically and systemically reduced using the GAL4-UAS system and an RNAi approach. Both KD-1 and KD-2 transgenic fly lines contained an inverted repeat sequence of the *Prx3* on chromosomes II and III, respectively. The RNAi effect became systemically active in all progeny following mating with the ubiquitous Gal4-expressed line (Act5C). Quantitative RT-PCR revealed that the expression levels of the *Prx3* in adult flies were significantly lower in the *Prx3* KD lines than in the control groups carrying Gal4 alone: the expression levels of the *Prx3* in the KD-1 and KD-2 mutants were 11% and 13% of those in the corresponding control lines, respectively (Fig. 2). We next investigated the resistance of the established *Prx3* KD lines against oxidative stress using 1–3-day-old male flies grown on agar plates containing 0.5% H₂O₂. These *Prx3* KD lines were phenotypically similar to the wild-type lines, and the developmental rate, sex ratio, and lifespan were also comparable between the knockdown and wild-type lines (data not shown). Moreover, the ectopic expression of the *Prx3* *in vivo* had no effect on lifespan (1). Under oxidative stress conditions, however, the median survival times of both KD lines was significantly lower than the control lines: the median

Fig. 2 Establishment of *Prx3*-specific knockdown lines. The fold RNA reductions of Act5C (ubiquitous promoter)-Gal4 > *Prx3*-anti1 (KD-1) and Act5C-Gal4 > *Prx3*-anti2 (KD-2) knockdown males are 11% and 13% compared to the control Act5C-Gal4/+ genotype (AG4), respectively. IR1 and IR2 are both transgenic fly lines that have the same *Prx3*-targeted RNAi construct but integrated chromosome of its construct is different (IR1; 2nd chromosome, IR2; 3rd chromosome). Total RNA was isolated from 40 adults of each genotype and purified using RNeasy RT (Molecular Research Center, INC.). First-strand cDNA was synthesized using ReverTra Ace reverse transcriptase (Toyobo, Osaka, Japan) with 2 µg of total RNA from each strain as a template and an oligo (dT) 20mer primer (Toyobo). Quantitative RT-PCR was performed on a ROTOR-GENE 6000 (Qiagen, Hilden, Germany) by following the manufacturer's instructions with SYBR green-based detection of PCR products. Data are expressed as mean ± SE values. Significant differences are denoted as **P* < 0.005. n = 5.

survival times of the KD-1 and KD-2 lines were 84% and 79% of the corresponding control lines, respectively (Fig. 3). The survival rates under the oxidative stress conditions declined with age (the number of days after eclosion). The survival curves of the 1–3-day-old *Prx3* KD lines were closer to those of the 10-day-old control lines than to those of the 1–3-day-old control lines.

These results suggest that the expression levels of the *Prx3* play a role in the oxidative stress response. The *Prx3* KD lines used in this study were more susceptible to oxidative stress, suggesting that Prx3 is involved in the oxidative stress response and the survival of *Drosophila* under drastic oxidative stress conditions. The control line Gal4/+ also showed decreased responsiveness to oxidative stress with age, similar to the results shown in Fig. 1. Our study also demonstrated that the responsiveness of the *Prx3* KD lines to oxidative stress became more closer to that of the older control lines, suggesting the involvement of Prx3 in aging-associated changes in the responsiveness to oxidative stress. Quantitative RT-PCR was performed to investigate the changes

Fig. 3 RNAi-induced *Prx3* knockdown mutants are sensitive to oxidative stress. **A.** Act5C (ubiquitous promoter)-Gal4 > *Prx3*-anti1 (KD-1) flies had a 16% shorter median lifespan than Act5C-Gal4/+ (AG4) controls ($P < 0.05$). The maximum survival time was reduced by 82%. IR1; *Prx3*-anti1/+ control. Sample sizes (males): KD-1, $n = 60$; IR1, $n = 57$; AG4, $n = 60$; 10 d AG4, $n = 30$. **B.** Act5C-Gal4 > *Prx3*-anti2 (KD-2) flies had a 21% shorter median lifespan than Act5C-Gal4/+ controls ($P < 0.005$). The maximum survival time was reduced by 82%. IR2; *Prx3*-anti2/+ control. Sample sizes (males): KD-2, $n = 95$; IR2, $n = 60$; AG4, $n = 60$; 10 d AG4, $n = 30$.

in the expression levels of the *Prx3* over time. It was revealed that its expression levels decreased in an age-dependent manner (Fig. 4). In particular, the expression of the *Prx3* in 30-day-old males was significantly lower than that in 5-day-old males. This result indicates that the expression levels of the *Prx3* may be crucial for the age-dependent competence of the oxidative stress response.

Human PRDX3 is a known target of the c-Myc oncogene (16). Although *Prx3* is ubiquitously expressed in cells throughout development, enhancers that respond to the presence of drastic oxidative stress may exist in age-dependent manner. Identifying such regulators of *Prx3* will clarify the detailed roles of *Prx3* in aging-associated changes in the re-

Fig. 4 The expression levels of *Prx3* are decreased in an age-dependent manner. Data represent the fold increase in *Prx3* mRNA levels, assessed using quantitative RT-PCR (5 d = 1, normoxia, standard food). The *Prx3* mRNA level was 75% less on day 30 than on day 5 post eclosion ($*P < 0.05$). RNA preparation and quantitative RT-PCR was performed as described in Fig. 2. Data are expressed as mean $\pm$ SE values. $n = 5$.

sponse to oxidative stress.

Acknowledgements

The following stocks were provided as generous gifts from S. Hirose and N. Fuse at the National Institute of Genetics, Japan: wild-type *Oregon-R*, *y¹ Df(1)w^{67c1}*; Actin5C-Gal4/TM6B (ubiquitous driver). The *Prx3* RNAi lines (*w¹¹¹⁸*; *5826R-1/SM1*, *w¹¹¹⁸*; *5826R-2*) were obtained from the NIG-FLY stock center. This work was supported by Grant-in-aid for Young Scientist (B) (24700837) (to Y.K.) and Scientific Research from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

REFERENCES

1. Anderson PR, Kirby K, Orr WC, Hilliker AJ and Phillips JP (2008) Hydrogen peroxide scavenging rescues frataxin deficiency in a *Drosophila* model of Friedreich's ataxia. *Proc Natl Acad Sci USA* **105**, 611–616.
2. Bulteau AL, Szweda LI and Friguet B (2006) Mitochondrial protein oxidation and degradation in response to oxidative stress and aging. *Exp Gerontol* **41**, 653–657.
3. Cox AG, Peskin AV, Paton LN, Winterbourn CC and Hampton MB (2009) Redox potential and peroxide reactivity of human peroxiredoxin 3. *Biochemistry* **48**, 6495–6501.
4. Fleming JE, Reveillaud I and Niedzwiecki A (1992) Role of oxidative stress in *Drosophila* aging. *Mut Res* **275**, 267–279.
5. Miller RL, James-Kracke M, Sun GY and Sun AY (2009) Oxidative and inflammatory pathways in Parkinson's disease. *Neurochem Res* **34**, 55–65.
6. Radyuk SN, Klichko VI, Spinola B, Sohal RS and Orr WC (2001) The peroxiredoxin gene family in *Drosophila melanogaster*. *Free Radical Biol Med* **31**, 1090–1100.
7. Radyuk SN, Sohal RS and Orr WC (2003) Thioredoxin peroxidases can foster cytoprotection or cell death in response

- to different stressors: over- and under-expression of thioredoxin peroxidase in *Drosophila* cells. *Biochem J* **371**, 743–752.
8. Rhee SG, Chae HZ and Kim K (2005) Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* **38**, 1543–1552.
 9. Ristow M and Zarse K (2010) How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp Gerontol* **45**, 410–418.
 10. Rossi P, Marzani B, Giardina S, Negro M and Marzatico F (2008) Human skeletal muscle aging and the oxidative system: cellular events. *Curr Aging Sci* **1**, 182–191.
 11. Sas K, Robotka H, Toldi J, and Vécsei L (2007) Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci* **257**, 221–239.
 12. Stadtman ER (2006) Protein oxidation and aging. *Free Radic Res* **40**, 1250–1258.
 13. Stroschein-Stevenson SL, Foley E, O'Farrell PH and Johnson AD (2006) Identification of *Drosophila* gene products required for phagocytosis of *Candida albicans*. *PLoS Biol* **4**, e4.
 14. Versari D, Daghini E, Viridis A, Ghiadoni L and Taddei S (2009) The ageing endothelium, cardiovascular risk and disease in man. *Exp Physiol* **94**, 317–321.
 15. Wei YH, Lu CY, Lee HC, Pang CY and Ma YS (1998) Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. *Ann NY Acad Sci* **854**, 155–170.
 16. Wonsey DR, Zeller KI and Dang CV (2002) The c-Myc target gene PRDX3 is required for mitochondrial homeostasis and neoplastic transformation. *Proc Natl Acad Sci USA* **99**, 6649–6654.