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BRIEF REPORT

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Impaired p53/CEP-1 is associated with lifespan extension through an age-related imbalance in the energy metabolism of *C. elegans*

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In the nematode *Caenorhabditis elegans*, the mammalian tumor suppressor p53 ortholog CEP-1 mediates the stress response, activates germ line apoptosis and regulates meiotic chromosome segregation. A reduction in its expression, which frequently occurs in mammalian cancer cells, extends lifespan and induces an adaptive response in *C. elegans*. However, these effects do not involve an increase in oxidative stress resistance. Here, we showed that intermittent exposure to hyperoxia, which induces oxidative stress resistance and lowers the production of ROS derived from mitochondrial respiration in *C. elegans*, slightly improved the lifespan extension of *cep-1* mutant. Interestingly, ATP levels were increased without an increase in oxygen consumption in *cep-1* mutant during aging. In the wild-type, lactate levels and consequentially the lactate/pyruvate ratio decreased during aging in adults. Furthermore, the expression levels of mitochondrial respiration-related *sco-1*, which is a target of p53/CEP-1, as well as those of gluconeogenesis regulation and mammalian sirtuin ortholog genes, were also increased in the aged and adaptive conditioned wild-type animals. In contrast, the lactate/pyruvate ratio increased in cells of the *cep-1* mutant and was amplified by intermittent hyperoxia. These results suggest that impaired p53/CEP-1 leads to an imbalance in the age-related energy metabolic alteration between mitochondrial oxidative phosphorylation and aerobic glycolysis and plays an important role in the extension of both intact and adaptive lifespans.

1 | INTRODUCTION

The mammalian p53 ortholog CEP-1 in the nematode *Caenorhabditis elegans* mediates the stress response, activates germ line apoptosis and regulates meiotic chromosome segregation (Derry, Putzke, & Rothman, 2001; Schumacher, Hofmann, Boulton, & Gartner, 2001). It is required for lifespan extension in adaptive responses to mild stress but a reduction in its expression, as occurs in mammalian p53-mutated cancer cells, increases lifespan in *C. elegans*. This does not involve resistance to oxidative stress (Ventura et al., 2009). In mammalian p53-mutated cancer cells, alteration of secondary metabolism is a phenomenon in which glycolysis is predominantly used for energy production and aerobic mitochondrial respiration, which involves the tricarboxylic acid (TCA) cycle and electron transport chain, is down-regulated (Warburg, 1956). The mammalian tumor suppressor p53 regulates mitochondrial oxygen consumption through transcriptional and post-transcriptional targets, such as the *SCO2* gene (Matoba et al., 2006), which encodes an assembly protein of cytochrome *c* oxidase (COX), the glycolytic enzyme phosphoglycerate mutase (PGM) gene (Kondoh et al., 2005) and a mitochondrial gatekeeper, the pyruvate dehydrogenase kinase (Pdk2) gene (Contractor & Harris, 2012).

An adaptive response to low doses of potentially harmful (e.g., chemical, thermal or radiological) agents can be induced in various organisms (Luckey, 1980). It can occur via not only direct exposure to the agents, but also aging-derived intracellular reactive oxygen species (ROS), and induces lifespan extension in *C. elegans* (Yanase, Yasuda, & Ishii, 2002). We previously found that exposure to

intermittent hyperoxia in *C. elegans* induces lifespan extension by regulating antioxidant and mitochondrial energy metabolism systems via insulin/insulin-like growth factor-1 (ins/IGF-1) signaling (Yanase & Ishii, 2008). p53/CEP-1 is closely linked to the adaptive response via mitochondrial respiration control (Torgovnick, Schiavi, Testi, & Ventura, 2010). However, it is still unclear how impaired p53/CEP-1 induces metabolic alterations in the cell. Here, we showed that impaired p53/CEP-1 functions in a manner similar to that of the metabolic alterations seen in mammalian cancer cells through transcriptional targets and leads to a lifespan extension and adaptive response.

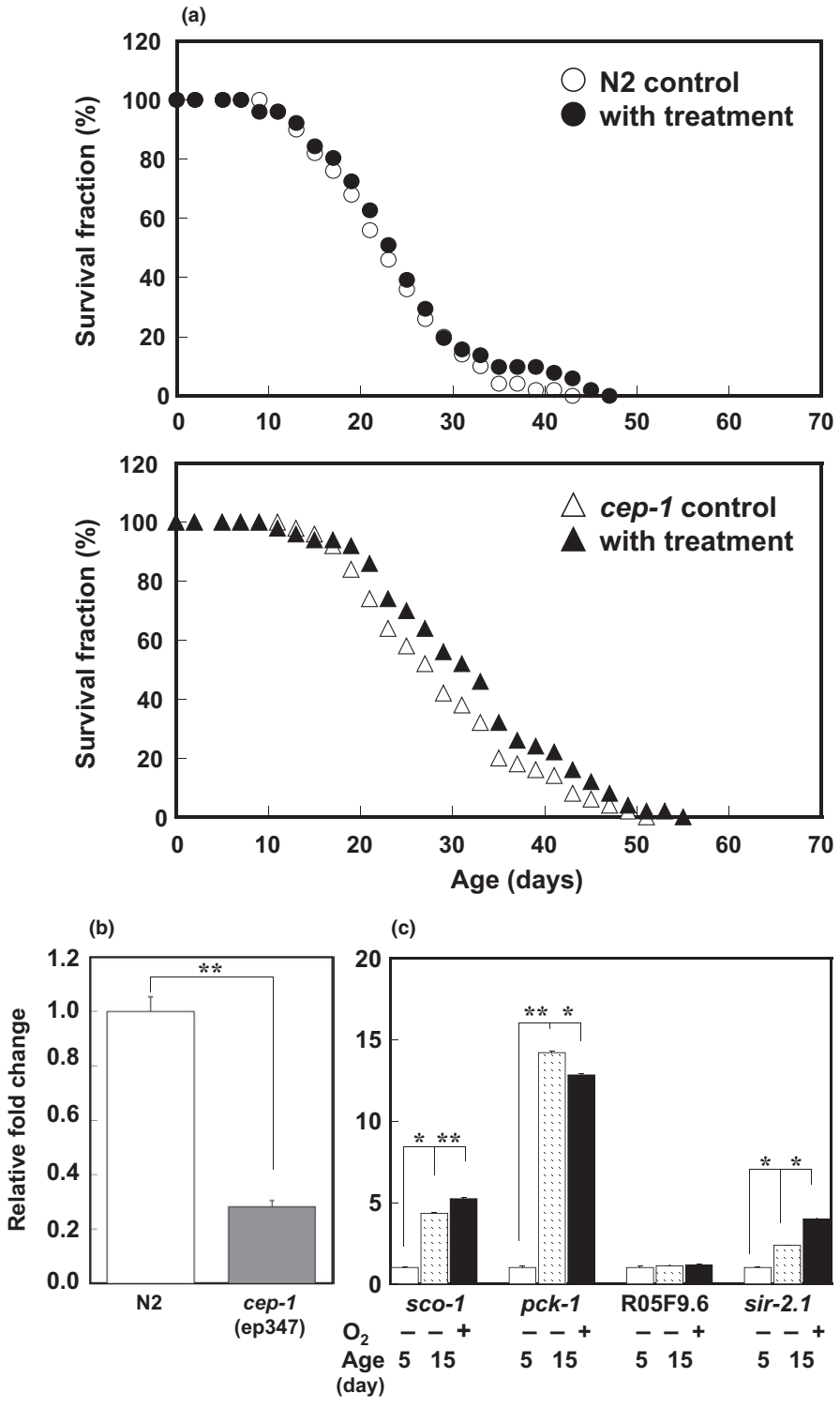

FIGURE 1 (a) Survival curves with or without the intermittent hyperoxia treatment. Mean lifespans ± *SD* were as follows; in wild-type with and without the exposure, 23.8 ± 1.0 days and 22.1 ± 1.1 days, in *cep-1* mutant with and without the exposure, 32.8 ± 4.5 days and 29.7 ± 4.0 days. Among mean lifespans of the *cep-1* mutant with or without the exposure and wild-type were compared using a *t* test ($p < .05$). (b) Real-time PCR data of the *sco-1* gene in 5-day-old wild-type and *cep-1* mutant. (c) Age- and hyperoxia-related changes in the mRNA levels of glycolytic genes in wild-type animals. Data are shown as the means ± *SEM*. Means were compared using a *t* test (*$p < .05$, **$p < .001$)

2 | RESULTS

2.1 | Lifespan extension in *cep-1* mutant induced by intermittent hyperoxia

The mean lifespan was increased (approximately 25%) in a deletion mutant lacking the DNA-binding domain of the *cep-1* gene compared with wild-type animals exhibiting normal aging. In addition, the mean lifespan of the *cep-1* mutant was slightly but significantly (approximately 10%) extended by intermittent exposure to hyperoxia (Figure 1a).

2.2 | Confirmation of p53/CEP-1 target and changes in other energy metabolism-related genes

To date, the mammalian *SCO1* and *SCO2* ortholog *sco-1* was only found in the *C. elegans* genomic DNA database (DDBJ/EMBL/GenBank). We identified the presence of potential p53/CEP-1 DNA-binding consensus sequences (El-Deiry, Kern, Pietenpol, Kinzler, & Vogelstein, 1992) −259/−239 bp upstream of the start codon (ATG) of the *sco-1* gene (data not shown). We examined that the *sco-1* gene expression levels were lower in *cep-1* mutant than in wild-type animals (Figure 1b).

In wild-type, aging and intermittent hyperoxia exposure increased the expression of the *sco-1* gene. Likewise, the *pck-1* gene encoding a phosphoenolpyruvate carboxykinase (known as PEPCK in mammals) that regulates gluconeogenesis (Yang, Kalhan, & Hanson, 2009), and the *sir-2.1* gene encoding a *C. elegans* sirtuin (also known as NAD^+-dependent histone deacetylase) that is induced upon caloric restriction (Tissenbaum & Guarente, 2001), showed increased expressions according to age and in response to hyperoxia. In contrast, expression of an ortholog gene (R05F9.6) of human phosphoglucomutase isozymes, which closely relate to the regulation of glycogen metabolism, was not changed under the conditions (Figure 1c).

2.3 | Changes in the ATP content and oxygen consumption rate in the *cep-1* mutant

By measuring adenosine triphosphate (ATP) content and oxygen consumption rates, we found that ATP levels were significantly higher in *cep-1* mutant than in wild-type, independently of age, whereas oxygen consumption rates were not significantly altered. In general, a decrease in ATP and/or an increase in AMP occur in cells as they age (Apfeld, O'Connor, McDonagh, DiStefano, & Curtis, 2004). In this study, the amount of ATP in *cep-1* mutant was not significantly different between 15-day-old and 5-day-old animals unlike the wild-type (Figure 2).

2.4 | Changes in intracellular lactate and pyruvate levels in *cep-1* mutant under intermittent hyperoxia

Intracellular lactate levels decreased in aged wild-type animals, and a consequent reduction in the lactate/pyruvate (L/P) ratio was seen. In contrast, intracellular lactate levels increased and pyruvate levels decreased in aged *cep-1* animals and those exposed to intermittent hyperoxia. Thus, the L/P ratio of *cep-1* mutant significantly increased according to age and in response to intermittent exposure to hyperoxia. These L/P ratio patterns appeared to be primarily caused by increased lactate or decreased pyruvate levels in the cells of aged *cep-1* mutant and of those exposed to intermittent hyperoxia (Figure 3a,b).

3 | DISCUSSION

In this study, we found that the intermittent hyperoxia extends the lifespan as an adaptive response in *cep-1* mutant. The lifespan extension during normal aging and the adaptive response can be attributed to metabolic alterations in

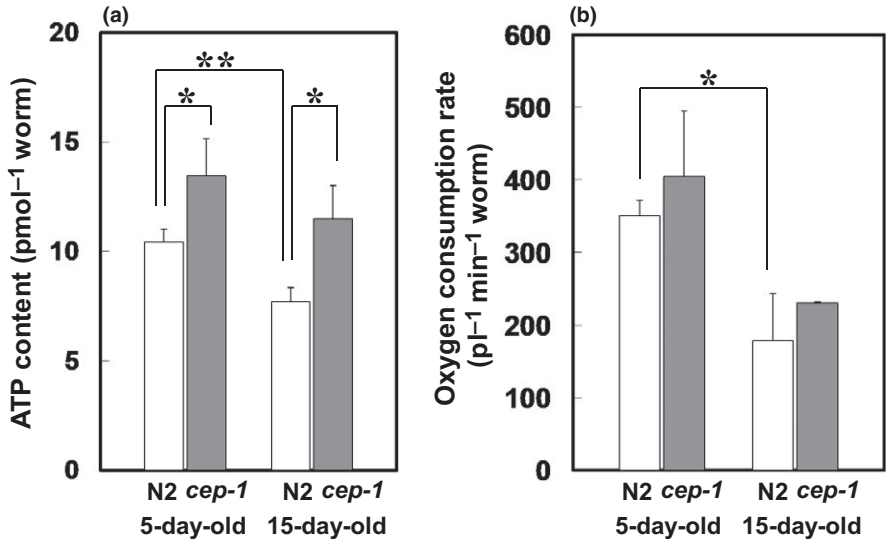

FIGURE 2 (a) ATP contents, (b) oxygen consumption rates in 5- and 15-day-old wild-type and *cep-1* mutant. Data are shown as the means ± *SD* of at least three independent experiments. Means were compared using a *t* test (*$p < .05$, **$p < .001$)

the impaired p53/CEP-1 cells. First, we examined that the expression levels of the *sco-1* gene, which is an ortholog of the mammalian *SCO1* and *SCO2* genes, were lower in *cep-1* mutant. Second, we examined that CEP-1 directly and indirectly regulates not only mitochondrial oxygen consumption but also glycolytic activity through the transcriptional targets such as mammalian p53 (Contractor & Harris, 2012; Kondoh et al., 2005; Matoba et al., 2006).

According to previous reports on energy metabolism in *C. elegans*, the TCA cycle is preferentially used for cell growth and proliferation during the L2 to L4 larval stages; subsequently, higher anoxia tolerance and greater survival are observed in young adult worms (Van Voorhies & Ward, 2000; Wadsworth & Riddle, 1989). The developmental characteristics of energy metabolism are associated with the invariant number of somatic cells, except adult germ cells, after cell division. The marked reductions in oxygen consumption and metabolic rates are seen in 10-day-old and older wild-type animals (Vanfleteren & De Vreese, 1996). Interestingly, these reductions are consistent with the gradual decaying muscle function seen in the adult stage, as shown by pharyngeal pumping and locomotion rates (Chow, Glenn, Johnston, Goldberg, & Wolkow, 2006). Here, we observed that the *sco-1* gene expression levels increased

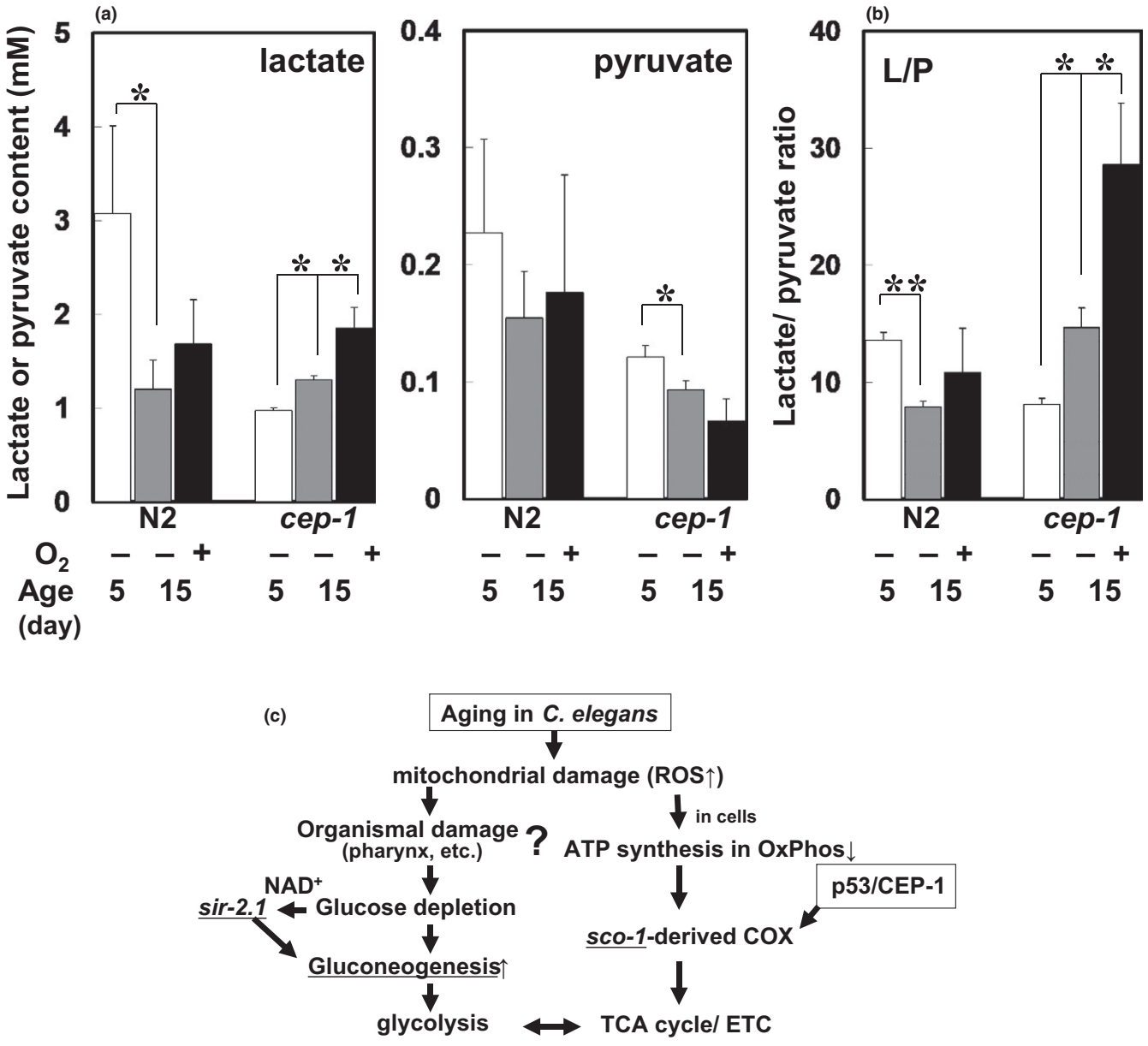

FIGURE 3 Changes in energy metabolism with aging and intermittent exposure to hyperoxia in wild-type and *cep-1* mutant. (a) Intracellular concentrations of lactate and pyruvate, (b) L/P ratios in 5- and 15-day-old wild-type and *cep-1* mutant. Data are shown as the means ± *SD* of three independent experiments. Means were compared using a *t* test (*$p < .05$, **$p < .001$). (c) Schema of the age-related energy metabolic imbalance and its association with p53/CEP-1

according to age in wild-type. However, ATP and oxygen consumption levels were significantly reduced in 15-day-old animals compared with 5-day-old animals, as previously reported (Suda, 2013; Suda, Shoyama, Yasuda, & Ishii, 2005; Vanfleteren & De Vreese, 1996). These data imply that mitochondrial components such as COX were damaged by mitochondrial ROS during the aging process, and the consequent aerobic glycolysis was used rather than the dominant usage of the TCA cycle (McElwee, Schuster, Blanc, Thornton, & Gems, 2006). Furthermore, the age-dependent increase in the expression levels of *pck-1* and *sir-2.1* genes indicates an acceleration of gluconeogenesis and calorie restriction during normal aging. Indeed, lactate levels and the consequent L/P ratio decreased in the aged wild-type. In contrast, the *cep-1* mutant showed increased ATP levels throughout aging compared with wild-type without increased oxygen consumption, as well as increases in lactate levels and the consequent L/P ratio depending on age and the adaptive condition. These results suggest the compensatory and preferential use of aerobic glycolysis to generate ATP instead of mitochondrial oxidative phosphorylation (OxPhos) in the impaired p53/CEP-1 mutant, which resembles the energy metabolism seen in mammalian cancer cells.

However, there is the important point of a unique metabolism related to the *pck-1* gene in invertebrates such as *C. elegans*. Although a free-living nematode, *C. elegans* also possesses the phosphoenolpyruvate carboxykinase (PEPCK)-succinate pathway, which is an anaerobic energy metabolism pathway in the mitochondria of the parasitic stage of *Ascaris* dwelling in the porcine gut (Rea & Johnson, 2003; Takamiya et al., 1999). *Caenorhabditis elegans* PCK-1 regulates several metabolic processes associated with cataplerosis, such as gluconeogenesis and PEPCK-succinate pathways in anaerobic conditions (Yang et al., 2009). Our results support the upregulation of gluconeogenesis during normal aging in wild-type, rather than the anaerobic metabolic pathway, due to the increased expression of *pck-1* and *sir-2.1* genes, reduction in aerobic respiration and decreased L/P ratio. Conversely, p53/CEP-1 supplements a component in COX damaged by mitochondrial ROS during normal aging through the target *sco-1* gene (Figure 3c). Therefore, we conclude that the impaired p53/CEP-1 leads to a metabolic imbalance during the aging process and mainly involves gluconeogenesis. It also has potential for the metabolic regulation of lifespan in mammalian nonregenerative tissues such as the somatic cells of *C. elegans*.

4 | EXPERIMENTAL PROCEDURES

4.1 | Nematode strains

The wild-type N2 strain var. Bristol and *cep-1(ep347)* (CE1255) strains of *C. elegans* were obtained from the Caenorhabditis Genetics Center at the University of Minnesota (Minneapolis, MN, USA). In the *cep-1* dysfunctional mutant, the *ep347* allele contains a deleted region of 1,827 base pairs (bp) at the C-terminus and middle of CEP-1 (the *cep-1* unspliced region, measuring 4,739 bp).

4.2 | Measurement of lifespans after intermittent hyperoxia treatment

Eggs were collected in utero by washing gravid hermaphrodites from nematode growth medium (NGM) agar plates and dissolving them in alkaline sodium hypochlorite. The released eggs were allowed to hatch during an overnight incubation at 20°C in S buffer without *Escherichia coli* to produce age-synchronous cultures of L1-stage larvae. The lifespan of the hermaphrodites at 20°C was measured using 100 worms per trial in at least three independent experiments. To prevent progeny production, 5-fluoro-2′-deoxyuridine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was added to the NGM agar plate to a final concentration of 40 μM after the animals had reached adulthood. The *C. elegans* strains were exposed daily to 90% oxygen for 3 hr in an airtight plastic chamber from 5 to 15 days of age (Yanase et al., 2002).

4.3 | Real-time PCR analysis of *sco-1* and other metabolism-related genes

The *C. elegans sco-1* gene is orthologous to the human *SCO1* gene, which has a "core" region with the highest sequence identity to the human *SCO2* gene and the yeast *SCO1* and *SCO2* genes. To amplify approximately 116 bp containing exons 2 and 3 using RT-PCR, the *sco-1* primers were established from nucleotide sequences corresponding to a region within the conserved amino acids of *SCO* and *SCO*-like polypeptides (Papadopoulou et al., 1999). In conjunction with analyses of other genes, quantitative measurements used a TaqMan gene expression assay in animals using a 7500 Fast Real-Time PCR System (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA, USA). The results were normalized to the transcript level of the *act-4* gene using the wild-type strain as the control.

4.4 | Measurement of ATP levels of individual worms

The ATP content of *cep-1* mutants exposed to intermittent hyperoxia was measured with a luciferase assay, performed as previously described (Suda, 2013). Bacteria attached to the bodies of the 5- and 15-day-old animals were removed by allowing the worms to crawl onto an NGM agar plate without OP50 for 30 min. Thereafter, each animal was transferred into 100 μl of M9 buffer including 0.001% SDS in a microtube to isolate ATP molecules from individual

worms. Microtubes were then sonicated using an ultrasonic homogenizer UH-50 (SMT Co., Ltd., Tokyo, Japan) for 30 sec. After the addition of 100 µl Extraction Reagent (TOYO B-Net Co., Ltd., Tokyo, Japan) to extract the ATP molecules, the luminescence (in counts per second) was subsequently measured by the addition of 100 µl of L/L Reagent using an AccuFLEX Lumi 400 luminometer (Aloka Co., Ltd., Tokyo, Japan).

4.5 | Measurement of oxygen consumption

The oxygen consumption rates of 5- and 15-day-old *cep-1* mutants were measured using 10 worms per trial. Oxygen concentrations were measured in a sample chamber (with a volume of 3.14 µl) in 5- and 15-day-old animals using a FOXY-2000 optical oxygen sensor (Ocean Photonics, Inc., Tokyo, Japan) calibrated at oxygen levels of 0% and normoxic levels of 20.95%. The 10 worms, which were allowed to crawl onto the NGM agar plates without a bacterial lawn to remove the live OP50 bacteria attached to their bodies, were carefully transferred into 0.7 µl of liquid medium (agar was omitted from the NGM) in the sample chamber. The liquid medium contained UV irradiation-killed OP50 bacteria (ca. 3×10^8 cells/ml). Each oxygen concentration measurement was taken within 40 min at room temperature (22–23°C) as previously described (Suda et al., 2005).

4.6 | Colorimetric assays of lactate and pyruvate

The *C. elegans* animals from each strain were synchronously cultured with or without intermittent exposure to hyperoxia. The surviving 5- and 15-day-old animals were separated from the dead using the sucrose method. After the animals were collected by centrifugation, the pellets were suspended in an equal volume of 10% trichloroacetic acid (TCA), homogenized using a Teflon homogenizer on ice and sonicated using an ultrasonic homogenizer UH-50 for 3 min. The homogenates were clarified by centrifugation at 10,000 *g* for 10 min. The supernatants were neutralized with 4 M KOH and centrifuged at 10,000 *g* for 10 min (Senoo-Matsuda et al., 2001). Concentrations of lactate and pyruvate were measured using two colorimetric assay kits, the Lactate Colorimetric/Fluorometric Assay Kit (BioVision Inc., Milpitas, CA, USA) and the EnzyChrom Pyruvate Assay Kit (BioAssay Systems, Hayward, CA, USA), respectively.

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