Chapter 9
Metabolic Biomarkers in Nematode *C. elegans* During Aging

Sumino Yanase, Takamasa Ishii, Kayo Yasuda, and Naoaki Ishii

Abstract Changes in energy metabolism occur not only in diseases such as cancer but also in the normal development and aging processes of various organisms. These metabolic changes result to lead to imbalances in energy metabolism related to cellular and tissue homeostasis. In the model organism *C. elegans*, which is used to study aging, an imbalance in age-related energy metabolism exists between mitochondrial oxidative phosphorylation and aerobic glycolysis. Cellular lactate and pyruvate are key intermediates in intracellular energy metabolic pathways and can indicate age-related imbalances in energy metabolism. Thus, the cellular lactate/pyruvate ratio can be monitored as a biomarker during aging. Moreover, recent studies have proposed a candidate novel biomarker for aging and age-related declines in the nematode *C. elegans*.

Keywords *C. elegans* · Energy metabolism · Aging · Metabolic change · Mitochondrial ROS · Cancer

S. Yanase (✉)
Department of Health Science, Daito Bunka University School of Sports & Health Science, Higashi-matsuyama, Saitama, Japan

Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan
e-mail: syanase@ic.daito.ac.jp

T. Ishii
Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan

K. Yasuda · N. Ishii
Department of Health Management, Undergraduate School of Health Studies, Tokai University, Hiratsuka, Kanagawa, Japan

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9.1 Introduction

In mammalian p53-mutated cancer cells, glycolysis is used predominantly for energy production while aerobic mitochondrial respiration is downregulated, a secondary metabolic change known as the Warburg effect [1]. Recent studies have revealed that the mammalian tumor suppressor p53 directly regulates oxygen consumption through transcriptional targets such as the *SCO2* gene, which encodes an assembly protein for the synthesis of cytochrome *c* oxidase (COX) in the mitochondrial respiration chain complex and is associated with changes in glycolytic activity in mice and human cancer cells [2]. Moreover, mammalian p53 regulates the glycolytic enzyme phosphoglycerate mutase (PGM) and the mitochondrial gatekeeper pyruvate dehydrogenase kinase (Pdk2) through post-transcriptional control [3, 4]. Likewise, we recently investigated whether the mammalian p53 ortholog CEP-1 in a model organism *Caenorhabditis elegans* (*C. elegans*) is associated with metabolic transition in the cells [5, 6]. Unlike tumorigenesis in mammals, impaired p53/CEP-1 extends the lifespan through an age-related imbalance in energy metabolism in *C. elegans* [7, 8]. The age-related imbalance in energy metabolism shows that lactate levels and consequently the lactate/pyruvate (L/P) ratio decrease during aging in wild-type adult. However, this phenomenon is different in *cep-1* mutants. Interestingly, changes in the L/P ratio during aging have also been observed in a mutant premature aging model in *C. elegans* [9]. Thus, classical energy metabolism and the inherent changes in metabolite levels are re-evaluated based on the cellular balance during aging and age-related diseases, and some metabolites could be highlighted as novel biomarkers [9, 10]. Here, we review the change in energy metabolism during aging in *C. elegans* and discuss the potential biomarkers in aging and age-related disorders in organisms.

9.2 Metabolism During Development and Aging in *C. elegans*

In the life cycle of *C. elegans*, there are normally four larval (L1-L4) and adult stages, as well as a facultative diapause ‘dauer’ larval stage. Dauer larvae do not feed despite being active [11, 12]. In the energy metabolism of *C. elegans*, the tricarboxylic acid (TCA) cycle is preferentially used for cell growth and proliferation during the L2 to L4 stages; subsequently, both higher tolerance to anoxia and greater protection against reactive oxygen species (ROS) are observed in young adult worms [12–14]. It is likely that the developmental characteristics of energy metabolism are associated with an invariant number of somatic cells, except the adult germ line, after somatic cell division. Marked reductions in oxygen consumption and metabolic rate have been seen in wild-type animals of 10-day-old and above [15]. These reductions are consistent with the gradual decay of muscle function seen in the adult stage, as revealed by pharyngeal pumping and locomotion rates [16, 17].

Recently, we showed that expression of the encoding mammalian COX assembly protein *SCO2* gene homolog *sco-1* gene increases according to age in wild-type *C. elegans* [8]. However, as previously reported, adenosine triphosphate (ATP) and oxygen consumption levels are significantly reduced during aging in the adult stages [15, 18, 19]. These results suggest that mitochondrial components, such as COX, are damaged by mitochondrial ROS during the aging process, and consequently aerobic glycolysis is utilized preferentially rather than the TCA cycle [20].

On the other hand, we showed the age-dependent increases in the expression levels of *pck-1* gene encoding a phosphoenolpyruvate carboxykinase (known as PEPCK or PCK1 in mammals), which regulates gluconeogenesis [21], and *sir-2.1* gene encoding a *C. elegans* sirtuin (also known as NAD^+-dependent histone deacetylase), which is induced upon caloric restriction [22, 23] in wild-type *C. elegans* [8]. These observations indicate an acceleration of gluconeogenesis and calorie restriction during normal aging. Indeed, lactate levels and the consequent L/P ratio decreased in aged wild-type *C. elegans*.

9.3 Hypoxia-Induced Metabolism and Aging in Nematode

The *pck-1* gene is related to an important role involved in unique energy production even in anaerobic environments during various life cycles of many parasitic invertebrates. Although *C. elegans* is a free-living nematode, it also possesses the PEPCK-succinate pathway, which is an anaerobic mitochondrial fermentation pathway for energy production during the parasitic stage of *Ascaris* species, which are mammalian intestinal roundworms [10, 24, 25]. In fact, starved and incubated *C. elegans* can normally survive for a few hours by utilizing carbohydrate stores under anoxic conditions [10, 26]. *C. elegans* PCK-1 regulates several metabolic processes associated with cataplerosis, which is the removal of intermediate metabolites from pathways, such as gluconeogenesis and PEPCK-succinate pathways in anaerobic environments [21, 27]. Our previous report also found the upregulation of gluconeogenesis rather than the anaerobic metabolic pathway during aging in *C. elegans* due to the increased expression of *pck-1* and *sir-2.1* genes, reduction in mitochondrial respiration, and decreased L/P ratio [8, 28].

9.4 Metabolic Changes in a Nematode Model of Premature Aging

According to the free radical theory of aging, the accumulation of ROS as by-products of mitochondrial metabolism is associated with lifespan determination and aging in various organisms [29, 30]. Almost all mitochondrial oxygen consumption is efficiently coupled to the production of ATP; however, a small part of the oxygen

consumed is reduced by wayward electrons to produce potentially toxic ROS. Indeed, mitochondria are the primary source of ROS in cells [31]. It is estimated that ~0.1% of the oxygen utilized by cells is only partially reduced and is leaked as a kind of ROS, e.g., superoxide anion ($O_2^{\cdot-}$) [32]. In *C. elegans*, lifespan is closely related to the concentration of environmental oxygen [33] and the continuous exposure to the hyperoxia accelerates senescence so that the levels of intracellular ROS increase in animals [34–36].

The *mev-1* gene encodes a large subunit of the enzyme succinate dehydrogenase cytochrome *b*, which is a component of complex II in the mitochondrial electron transport chain. Mutation of the gene causes an increase in mitochondrial $O_2^{\cdot-}$ production and consequently shortenes the lifespan of *C. elegans* [37]. Mitochondrial electron transport chain transfers reducing equivalents from NADH (in complex I) and $FADH_2$ (in complex II) in the form of electron flow through complexes III and IV. In the four complexes, which are connected with CoQ and cytochrome *c* (Cyt *c*), protons (H^+) are pumped from the matrix into the intermembrane space to establish an electrochemical gradient and subsequently drive ATP synthase to generate ATP. When flow to oxygen as the final acceptor of electrons is restricted because of a higher H^+ gradient or inhibition at the complexes, the chance of electron leakage increases. The leaked electrons from complexes I and III are transferred to molecular oxygen in the mitochondria and consequently cause the generation of $O_2^{\cdot-}$. Mitochondrial manganese superoxide dismutase (Mn SOD) catalyzes the conversion of $O_2^{\cdot-}$ into hydrogen peroxide (H_2O_2) in the matrix (Fig. 9.1) [38]. In *mev-1* mutant, $O_2^{\cdot-}$ production increases at complex I rather than at complexes II and III, and the lactate levels and L/P ratio are markedly higher than in the wild-type [9].

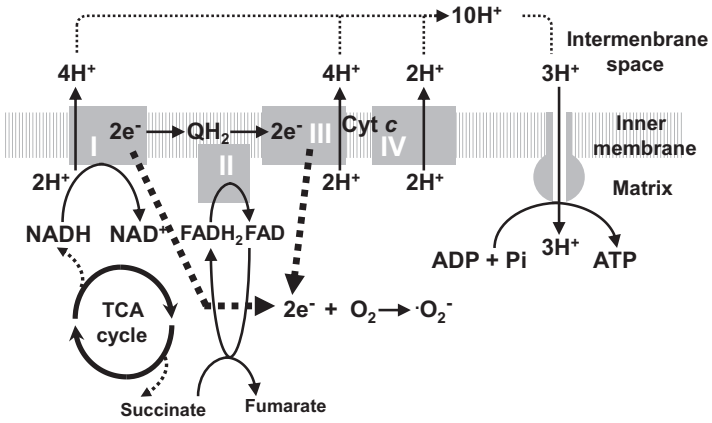

Fig. 9.1 Schematic of electron and H^+ transport in the electron transport chain of mitochondrial OxPhos. Four complexes are connected to CoQ, Cyt *c* and other molecules included in the chain. Electrons leak mainly from complexes I and III during the higher H^+ gradients

These observations suggest that *mev-1* animals preferentially utilize glycolysis instead of using the TCA cycle and subsequently the electron transport chain in mitochondria for production of cellular energy. Interestingly, a recent study has demonstrated that p53/CEP-1 inactivation rescues the shortened lifespan of the *mev-1* mutant [39]. Despite overproduction of ROS by mitochondria in the *mev-1* mutant, however, we observed increases in the expression of COX assembly protein-encoding *sco-1* gene, which is a target of p53/CEP-1 and associated with regulation of mitochondrial respiration (unpublished data). Thus, the levels of cellular metabolites such as lactate and pyruvate, which are key intermediates in the cellular energy metabolic pathways, correlate with a switch from mitochondrial respiration to glycolysis in energy metabolism during aging [8, 9].

9.5 Metabolism of Mutants Related to the Longevity in *C. elegans*

9.5.1 Metabolism in Reduced ins/IGF-1 Signaling

Intracellular ROS levels are regulated via an insulin/insulin-like growth factor-1 (ins/IGF-1) signaling pathway [40, 41], which determines longevity and resistance to oxidative stress in *C. elegans* [42–44]. Through the DAF-16 transcription factor [45, 46], which is the *C. elegans* homolog of the mammalian forkhead transcription factor class O (FoxO) and activated downstream of the ins/IGF-1 signaling pathway, target genes such as those related to antioxidants, mitochondrial respiration, and protein repair systems are regulated during normal aging (Fig. 9.2) [47, 48]. Thus, not only antioxidant systems containing SOD and catalase but also intracellular ROS levels containing mitochondrial $O_2^{\cdot-}$ are modulated via the ins/IGF-1 signaling pathway and are related to determining the lifespan of *C. elegans* [35, 43, 44, 52].

Many previous studies have demonstrated that the quantity of ATP production in both long-lived mutants with genes *age-1* (encoding a homolog of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) and *daf-2* (encoding a homolog of the mammalian insulin receptor) [40, 41, 53], which inactivates ins/IGF-1 signaling, is increased compared with wild-type *C. elegans* [45, 54–57]. In addition, the mutants consistently show lower respiratory rates despite increased ATP concentrations [58–62]. That is, a reduction in the metabolic rate is required to extend the lifespan of *C. elegans* [60]. These observations suggest the possibility that worms with reduced ins/IGF-1 signaling preferentially utilize aerobic glycolysis and gluconeogenesis through glyoxylate shunt, which is not present in mammals, rather than mitochondrial respiration in the cells [20]. Intracellular ROS as by-products of the ATP production process in the mitochondrial respiratory chain are efficiently removed due to the higher activities of antioxidant enzymes such as SOD and catalase in *age-1* and *daf-2* mutants [58, 63].

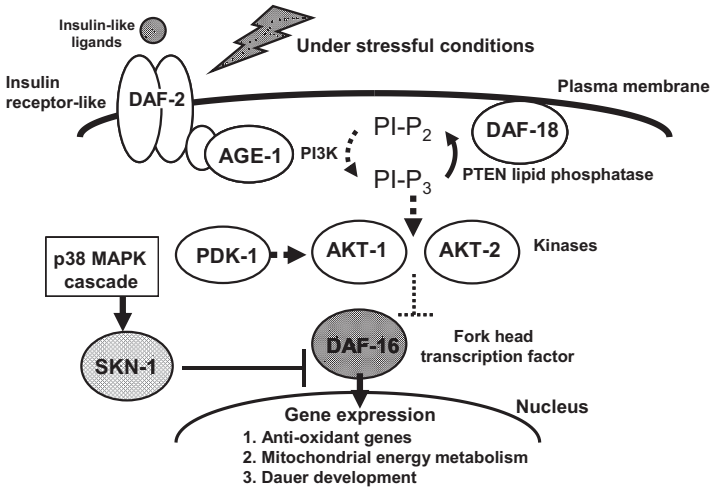

Fig. 9.2 Schematic model of the ins/IGF-1 signaling pathway associated with aging in *C. elegans*. As shown in the schematic, other signals, for instance mammalian NF-E2-related factor (Nrf) ortholog SKN-1 up-regulated by the p38 mitogen-activated protein kinase (MAPK) signaling pathway, plays an important role in fine-tuning molecular compensation among *sod*-genes during normal aging [49–51]

9.5.2 Metabolism Related to Mitochondrial Respiratory Chain

Mutations of the *clk-1* gene (encoding a homolog of the yeast COQ7/CAT5, a component of mitochondrial respiratory chain) in *C. elegans*, show slowing of developmental and physiological processes, including growth, pharyngeal pumping rate, defecation cycle and aging [64]. In the yeast *Saccharomyces cerevisiae*, mutations of the *coq7* gene prevent the biosynthesis of ubiquinone (coenzyme Q or CoQ), a lipid-soluble component of the electron transport chain required for mitochondrial respiration and gluconeogenesis [65, 66]. The mildly reduced mitochondrial respiration of long-lived *clk-1* mutants in *C. elegans* suggests that longevity is promoted by an age-dependent decrease in mitochondrial function [55, 67]. However, aged *clk-1* mutants also retain substantial elevation in ATP levels compared with wild-type animals. Interestingly, energy production and oxygen consumption appear to be uncoupled in *clk-1* mutants [55]. Dietary withdrawal of coenzyme Q from *Escherichia coli* (*E. coli*) extends the lifespan of not only wild-type but also *clk-1* mutant adults [68]. Similar results were reported in COQ7-deficient mice [69]. These findings suggest that mitochondrial coenzyme Q regulates the coupled mitochondrial respiration and generation of ROS that substantially contribute to the lifespan extension in various aerobic organisms.

9.5.3 p53/CEP-1 Dependent Energy Metabolic Regulation

In contrast, the *C. elegans cep-1* mutant shows increased ATP levels throughout aging without increased oxygen consumption compared with wild-type, as well as increases in lactate levels and the consequent L/P ratio depending on age. These results suggest the compensatory and preferential use of glycolysis to generate ATP rather than mitochondrial oxidative phosphorylation (OxPhos) related to the impaired p53/CEP-1, which resembles the energy metabolism seen in mammalian cancer cells. In addition, this implies that the unique anaerobic metabolism related to the PCK-1 in *C. elegans* activates the generation of ATP, as described in Sect. 9.3 [8]. Wild-type p53/CEP-1 supplements a component in COX, which is damaged by mitochondrial ROS during normal aging, through the target *sco-1* gene encoding a COX assembly protein in *C. elegans*. Moreover, a recent report shows that p53 with SIRT6 regulates gluconeogenesis by the promoting of nuclear exclusion of FoxO1 transcription factor, which mediates the activation of PCK1 [70]. Therefore, we conclude that impaired p53/CEP-1 leads to a metabolic imbalance during the aging process and mainly involves PCK1-mediated gluconeogenesis. It also has the potential for metabolic regulation of lifespan in mammalian post-mitotic cells after differentiation, for example, in somatic cells of *C. elegans* [8].

9.6 Homeostatic Control of Energy Balance in Caloric Restriction

Caloric restriction (CR) and fasting has been shown to extend lifespan and postpone age-related decline in various organisms from yeast to mammals. Indeed, *C. elegans* mutants with a slower pharyngeal pumping rate (e.g., *eat-2*, *clk-1* mutants) and a reduced bacterial food intake live significantly longer than the wild-type. The long lifespans of animals with mutations in the *eat-2* gene, which encodes a nicotinic receptor subunit, do not require the activity of DAF-16 transcription factor downstream of ins/IGF-1 signaling, and show no reduction in metabolic rate [71, 72]. Moreover, several recent studies revealed that a few transcription factors in *C. elegans* are closely associated with these phenomena (Fig. 9.3) [73, 74]. CR activates a mammalian basic leucine zipper transcription factor NF-E2-related factor 2 (Nrf2) homolog SKN-1, which signals the peripheral tissues to increase metabolic activity in a pair of ASI neurons in the head of *C. elegans* [73]. The transcription factor PHA-4, which is a homolog of the mammalian FoxA transcription factors family, has an important role in regulating the expression of superoxide dismutases-encoding *sod*-genes in head and tail neurons and intestinal cells, particularly in response to fasting, and lead to the regulation of glucagon production and glucose homeostasis [74]. Thus, mitochondrial ROS production as a trade-off for a temporary higher metabolic rate in peripheral cells consequently induces the expression of

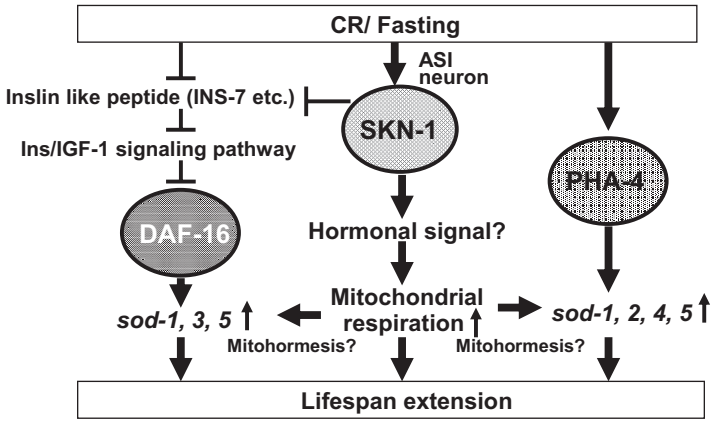

Fig. 9.3 Schematic model of crosstalk of transcriptional factors related to caloric restriction in *C. elegans*

sod-genes, and ultimately enhances longevity-mediated 'mitohormesis' in *C. elegans*.

Mitochondrial hormesis, or more simply mitohormesis, is a hypothetical concept that involves hormetic extension of lifespan [75]. In *C. elegans*, reduced glucose availability promotes ROS production as a side effect of the mitochondrial respiration, and causes induced antioxidant activity, thus increasing oxidative stress resistance and lifespan. The lifespan extension due to reduced glucose is abolished in the disruption of the *aak-2* gene, which encodes a homolog of mammalian AMP-activated protein kinase (AMPK) in *C. elegans*. AMPK activation leads to a decrease in the mammalian target of rapamycin (mTOR) activity [76]. Therefore, AAK-2 activated by a higher AMP/ATP ratio in cells under CR conditions, such as reduced glucose, functions independently of the ins/IGF signaling pathway to extend lifespan due to the decrease in mTOR signaling [77].

On the other hand, recent studies have proposed the hypothesis that CR extends lifespan at least in part by increasing the levels of ketone bodies in various organisms, including nematodes and rodents [78]. The ketone bodies, β-hydroxybutyrate (βHB) and the oxidized forms, which were first found in the ketonuria in diabetes mellitus, are produced by a reversal of the β-oxidation pathway in the metabolism of fatty acids and also during reduced carbohydrate intake such as starvation and fasting. Feeding βHB, which is a histone deacetylase (HDAC) inhibitor, extends the lifespan of *C. elegans* depending on the longevity signals of ins/IGF-1 and p38 MAPK cascade [79]. Thus, reduced glucose intake induces activation of alternative energy metabolic pathways and subsequently changes in the levels of cellular metabolites containing ketone bodies, which are related to longevity. Therefore, the cellular levels could act as metabolic biomarkers to enable understanding of individual energy conditions associated with aging and age-related decline.

9.7 Conclusions

Several genetic mechanisms of aging and lifespan have been clarified to date using the nematode *C. elegans* and are consistent, at least in part, among various organisms. However, the aging process is highly complex due to crosstalk between genetic signaling pathways. Recently, the various roles of mitochondria in the several signaling pathways associated with age-related functions, including energy metabolism, free radical production and apoptosis in aerobic organisms, have been specifically highlighted. In addition, mitochondrial dysfunction during aging is a trigger that induces many age-related changes in energy metabolism. Therefore, identification of these changes among cellular metabolites could help to estimate the condition of an individual and classify age-related disorders such as cancer, diabetes, sarcopenia, and neurodegenerative diseases in aging humans. The cellular levels of these metabolites could be used as genetic-dependent metabolic biomarkers to understand the individual energy conditions associated with aging and age-related declines. The unique ability of energy metabolism pathways in *C. elegans* might function under both aerobic and anaerobic conditions during aging. The use of model organisms contributes to our understanding of not only the mechanisms of aging but also cellular metabolic changes during aging and age-related decline in health.

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