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ORIGINAL CONTRIBUTION

Sesamin extends lifespan through pathways related to dietary restriction in *Caenorhabditis elegans*

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Abstract
Purpose Sesamin, a polyphenolic compound found in sesame seeds, has been reported to exert a variety of beneficial health effects. We have previously reported that sesamin increases the lifespan of *Caenorhabditis elegans*. In this study, we investigated the molecular mechanisms underlying the longevity effect of sesamin in *C. elegans*.
Methods Starting from three days of age, *Caenorhabditis elegans* animals were fed a standard diet alone or supplemented with sesamin. A *C. elegans* genome array was used to perform a comprehensive expression analysis. Genes that showed differential expression were validated using real-time PCR. Mutant or RNAi-treated animals were fed sesamin, and the lifespan was determined to identify the genes involved in the longevity effects of sesamin.
Results The microarray analysis revealed that endoplasmic reticulum unfolded protein response-related genes, which have been reported to show decreased expression under conditions of SIR-2.1/Sirtuin 1 (SIRT1) overexpression, were downregulated in animals supplemented with sesamin. Sesamin failed to extend the lifespan of *sir-2.1* knockdown animals and of *sir-2.1* loss-of-function mutants. Sesamin was also ineffective in *bec-1* RNAi-treated animals; *bec-1* is a key regulator of autophagy, and is necessary for longevity induced by *sir-2.1* overexpression. Furthermore, the heterozygotic mutation of *daf-15*, which encodes the target of rapamycin (TOR)-binding partner Raptor, abolished lifespan extension by sesamin. Moreover, sesamin did not prolong the lifespan of loss-of-function mutants of *aak-2*, which encodes the AMP-activated protein kinase (AMPK).
Conclusions Sesamin extends the lifespan of *C. elegans* through several dietary restriction-related signaling pathways, including processes requiring SIRT1, TOR, and AMPK.

Keywords Sesamin · Lifespan · *Caenorhabditis elegans* · Dietary restriction

Introduction

Dietary restriction (DR) or caloric restriction (CR) extends lifespan in a wide variety of animals [1]. This concept was first shown in rodents [2], and subsequent studies using monkeys showed that CR delays age-related mortality [3] and all-cause mortality [4]. A parallel study conducted at the National Institute of Aging showed that modest benefits in overall measures of health and function were observed in CR monkeys, although a significant difference in survival was not detected between control-fed and CR animals ($p = 0.06$) [5]. DR also exerts longevity effects in invertebrates such as fruit flies [6] and the nematode *Caenorhabditis elegans* [7, 8].

CR-mediated lifespan extension has been intensively studied in yeast. Lin et al. reported that CR (limiting

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glucose availability) extends the lifespan (the number of mother cell divisions) of this organism via a Sir2-dependent pathway [9]. This protein is a member of the sirtuins, a family of nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases [10]. However, it remains controversial whether Sir2 acts as a master regulator of CR-mediated lifespan extension [11]. In *C. elegans, eat-2* weak mutant worms, which represent a genetic DR model due to insufficient food intake, exhibit *Sir2* homolog (*sir-2.1*)-dependent lifespan extension [12], although several other studies have reported that DR extends lifespan through *sir-2.1*-independent mechanisms [13, 14]. Lifespan extension caused by DR in fruitflies also requires *Sir2* [15]. In mammals, seven sirtuins (SIRT1-7) have been identified and multiple lines of evidence have indicated that sirtuins mediate the effects of CR in mammals [16]. For example, knockout mice studies have shown that increases in physiological activities during CR and the increased longevity induced by CR require SIRT1 [17–19]. CR induces the expression of a subset of sirtuins in mice [20–22] and also in human [23].

Although DR provides beneficial effects, the process could cause adverse side effects such as decreased fertility [24]. Candidate DR mimetics that provide the beneficial effects of DR through similar mechanisms without inducing undesirable consequences have been reported [24]. For instance, resveratrol extends the lifespan of nematodes and fruit flies in a *sir-2.1*/*sir2*-dependent manner without reducing fertility [25]. Resveratrol's effects on lifespan are, however, less clear in mammals; for instance, although resveratrol prolongs the lifespan of obese mice [26], no evidence exists concerning longevity effects in non-obese mammals [27]. In mice, resveratrol delays age-related deterioration but does not extend lifespan [28].

Sesame seeds contain a class of propyl phenolic dimers known as lignans. Sesamin is one of the most abundant lignan in sesame seeds [29]. Sesamin has been reported to mediate biological effects such as the reduction of IgE production [30], inhibition of carcinogenesis [31], suppression of hypertension [32], and reduction of cholesterol levels [33] and fatty acid synthesis [34]. We have previously shown that sesamin supplementation extends lifespan in *C. elegans* [35]. *Caenorhabditis elegans* has been used extensively as an experimental animal model, especially for studies on senescence and the influence of food and nutrition. The appeal of this organism is due to its ease of cultivation, an abundance of genetic tools, and the short and reproducible lifespan [36].

In the present study, we examined the molecular mechanisms underlying the longevity effect of sesamin in *C. elegans*. Based on the results from microarray analyses that support the involvement of *sir-2.1*, we showed that several DR-related pathways mediate the effect of sesamin.

Materials and methods

Chemicals

Sesamin was purchased from Wako (Osaka, Japan). γ-Cyclodextrin (γCD) was obtained from Cyclochem (Kobe, Japan).

Bacterial strain and culture conditions

Escherichia coli OP50, which is used as the standard feed for *C. elegans* cultivation, was grown on tryptone soya agar (Nissui Pharmaceutical, Tokyo, Japan) at 37 °C. Cultured bacteria were scraped and weighed. Aliquots (100 mg wet weight) of bacteria were suspended in 0.5 ml M9 buffer (5 mM potassium phosphate, 1 mM $CaCl_2$, 1 mM $MgSO_4$), and used in the experimental assays.

Caenorhabditis elegans strains and culture conditions

The wild-type *C. elegans* strain Bristol N2 and its derivative mutant strains were obtained from the Caenorhabditis Genetics Center as follows: VC199 *sir-2.1(ok434) IV*, DR412 *daf-15(m81)/unc-24(e138) IV*, and RB754 *aak-2(ok524) X*. *Caenorhabditis elegans* strains were maintained using standard techniques [37]. For gene expression analyses and lifespan assays, animals were cultured as follows: eggs were prepared from adult *C. elegans* by exposure to a sodium hypochlorite/sodium hydroxide solution. The egg suspension was incubated in M9 buffer for one day at 25 °C to allow hatching and synchronization, and the resulting suspension of synchronized L1-stage worms was centrifuged at 156×*g* for 1 min. After removing the supernatant by aspiration, the remaining larvae were transferred onto mNGM plates (1.7% (w/v) agar, 50 mM NaCl, 1 mM $CaCl_2$, 5 μg/mL cholesterol, 25 mM KH_2PO_4, 1 mM $MgSO_4$) covered with 10 mg OP50. Transferred worms were cultured at 25 °C for two days until worms reached the young adult stage (referred to as 3-day-old animals).

Administration of sesamin

Sesamin was administered in the form of a γCD inclusion complex so that *C. elegans* could ingest the compounds; the complex was prepared and administered as previously described [35, 38] but with some modifications. Briefly, 500 μl sterile γCD water solution (230 mg/ml) was mixed with 50 μl sterile sesamin ethanol solution (2.5 mg/ml) and stirred with a rotary mixer for 12–24 h at ambient temperature. The resulting solid complex (inclusion complex) was collected by centrifugation, followed by suspension in 125 μl M9 buffer. An aliquot (12.5 μl) of this sesamin-γCD inclusion complex suspension was adjusted to 100 μl

by the addition of M9 buffer, and the diluted complex was combined with 100 µl OP50 suspension. The resulting suspension was spread onto mNGM plates (100 µl/plate). The amount of sesamin in the sesamin-γCD inclusion complex was determined as follows: the complex was prepared from 300 µl sesamin solution and 3 ml γCD solution, freeze-dried, and weighed (4.0 mg). The freeze-dried complex was mixed with dimethyl sulfoxide (DMSO)-d_6, and the nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AVANCE III HD 600 spectrometer (600 MHz for ^{1}H). The ^{1}H-NMR spectra were obtained at 25 °C. To obtain quantitative information through integral-based calculations, the ^{1}H experiments used a 90° pulse, non-spinning mode, and 60 s for the relaxation delay D1. NMR chemical shifts were referenced to the solvent peak δ_H 2.49 (residual DMSO-d_6). The NMR spectra for sesamin (Online Resource Fig. S1) and γCD (Online Resource Fig. S2) were used for the assignment of signals. From the integrated intensity of each signal in the spectrum of the sesamin-γCD inclusion complex (Online Resource Fig. S3), the molar ratio of sesamin to γCD in the inclusion complex was calculated to be approximately 1:1.36, namely, 4.0 mg sesamin-γCD inclusion complex contained approximately 669 µg sesamin, and one assay plate was considered to be supplemented with 5.75 µg sesamin.

Microarray analysis

Three-day-old worms were cultured for one day on mNGM plates covered with OP50 alone (control-fed group) or with OP50 supplemented with sesamin (sesamin-fed group). Microarray expression profiling was performed with control-fed and sesamin-fed worms. Approximately, 100 worms in each group were collected by a worm picker and soaked in RNA*later* solution (Qiagen). Total RNA was isolated using the RNeasy Lipid Tissue kit (Qiagen).

DNA synthesis and microarray hybridization were performed by Kurabo Industries Ltd. RNA quality (RNA integrity number (RIN) > 7) was confirmed using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). A total of ~1 µg of RNA was used as the template for fluorescent labeling of cRNA. Labelled cRNAs were hybridized to the Affimetrix *C. elegans* Genome Array (containing 22,500 transcripts). Microarray data analyses were performed with the MAS5.0 (Microarray Suite statistical algorithm, Affymetrix). Differential expression was analyzed by the Comparison Analysis of MAS5.0 using the Wilcoxon's Signed Rank test. Each probe set on the experiment array (sesamin-fed group) was compared to its counterpart on the baseline array (control-fed group), and a 'Change *p* value' was calculated. Probe sets that showed differential expression were assigned with 'Change calls' (Increase ($p < 0.002$), Marginal Increase ($0.002 \leq p < 0.002667$), Marginal Decrease ($0.997333 < p \leq 0.998$), or Decrease ($p > 0.998$)). The 'Signal Log Ratio' was computed using a one-step Tukey's Biweight method by taking a mean of the log ratios of probe pair intensities across the two arrays (control-fed vs. sesamin-fed group). The final data extraction was performed using DNA Microarray Viewer ver. 2 (Kurabo Industries, Ltd., Osaka, Japan).

Reverse transcription and real-time PCR

Genomic DNA was removed and cDNA was synthesized using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR (quantitative PCR) was performed in a StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA) using FastStart Universal SYBR Green Master (ROX) (Roche). All data were normalized to the *cyc-1* gene. Samples from four independent replicates were analyzed. The primers used for real-time PCR were as follows: *cyc-1*, (F: Forward) 5′-CGTGGTTCAAGGATC TAAACG-3′ and (R: Reverse) 5′-ACCGAGTTCTCCAAA GCGTA-3′; *abu-7*/*abu-8*, (F) 5′-CGACAACTCCTGCAC ATCC-3′ and (R) 5′-GTAAGTTGGCTGGGCTTGTT-3′; *abu-10*, (F) 5′-CCAACAATCCCAACATTCGT-3′ and (R) 5′-TTGGCATACGCATTGGTTAG-3′; *abu-13*, (F) 5′-CTT GTTCAGCCAGTCATTCG-3′ and (R) 5′-CCATTAGCT TTGTTAAATTCTGTGG-3′; *abu-14*, (F) 5′-CGCTGA CGAAGAGACTGTCA-3′ and (R) 5′-CGCAGCATGAGT TGGAGTT-3′; *pqn-32*, (F) 5′-CAGAGACCACAGGTT CAGCAC-3′ and (R) 5′-GCTGCAGTGGGATGTTGA-3′; *pqn-73*, (F) 5′-ATTTCCCGCATTGGATCAT-3′ and (R) 5′-CCTGATTAGGCCCACTTCC-3′.

Determination of *C. elegans* lifespan

Lifespan assays were performed as follows: synchronized 3-day-old animals (35 animals per plate) were placed on 5 cm mNGM plate covered with 10 mg OP50 alone or with sesamin supplementation and the plates were incubated at 25 °C. Animals were transferred daily to fresh plates for the first four days and thereafter transferred every second day. The numbers of live and dead animals were scored every day. An animal was considered dead when it failed to respond to a gentle touch with a worm picker. Animals that crawled off the plate or died from internal hatching were considered lost and not included in the analysis. Each assay was conducted in duplicate and repeated twice, except for the assays using *sir-2.1* mutants (repeated three times). Worm survival was calculated by the Kaplan–Meier method, and survival differences were tested for significance using the log-rank test.

Mean lifespan (MLS) was estimated using the formula [39]

$$\mathrm{MLS} = \frac{1}{N}\sum_{j}\frac{x_j + x_{j+1}}{2}d_j,$$

where d_j is the number of worms that died in the age interval (x_j to x_{j+1}) and N is the total number of worms. The standard error (SE) of the estimated mean lifespan was calculated using the following equation:

$$\mathrm{SE} = \sqrt{\frac{1}{N(N-1)}\sum\left(\frac{x_j + x_{j+1}}{2} - \mathrm{MLS}\right)^2 d_j}.$$

Maximum lifespan was calculated as the mean lifespan of the longest living 15% of the worms in each group.

RNAi experiments

RNA interference (RNAi) was carried out by feeding animals dsRNA-producing bacteria as previously described [40] but with some modifications. Animals were treated by RNAi over two generations to ensure that knockdown effects were stable and efficient. Briefly, synchronized L1-stage worms were transferred to plates containing RNAi-bacteria grown on NGM containing 50 µg/mL ampicillin and 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG), and the plates were incubated for 2 days at 25 °C until the transferred animals developed into young adults (P0). Eggs were collected from P0 animals and synchronized L1-stage larvae (F1) were prepared. F1 animals were cultivated for 2 days at 25 °C under RNAi conditions until the worms developed into young adults (3-day-old animals), at which point the animals were used in the following assays.

Measurement of ATP

Three-day-old worms were cultured for six days on mNGM plates covered with OP50 alone or with OP50 supplemented with sesamin. Approximately, 400 worms in each group were harvested into 1 ml M9 buffer. Worm pellets were washed four times with M9 buffer, resuspended in lysis buffer and immediately frozen in liquid nitrogen. The frozen pellets were boiled for 15 min at 100 °C to release ATP and dilution buffer was added. Samples were centrifuged at 15,000×*g* for 5 min. The supernatant was diluted with dilution buffer and used for measurement of ATP with the ATP Bioluminescence Assay Kit HSII (Roche) according to the manufacturer's instructions. For normalization of the luminescence signal, protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Dietary restriction protocol

Synchronized 3-day-old animals were cultured at 25 °C on mNGM plates covered with 5 mg OP50 for the first two days (*ad libitum* conditions), 0.05 mg OP50 for the next three days (DR conditions), and 0.1 mg OP50 (DR conditions) thereafter. Animals were transferred daily to fresh plates for the first five days and subsequently transferred every second day.

Results

Microarray expression profiling for genes regulated by sesamin

To identify genetic pathways that may contribute to sesamin-mediated lifespan extension, we performed a microarray analysis. Comparing the transcriptional profiles of control-fed and sesamin-fed worms, 13 upregulated genes (log-fold change ≥1) and 217 downregulated genes (log-fold change ≤1) were identified (Online Resource Table S1, S2). Functional annotation using DAVID (https://david.ncifcrf.gov/summary.jsp) revealed that the enriched gene ontology (GO) terms for genes downregulated by sesamin supplementation frequently indicated 'development' or 'morphogenesis' (Table 1). 'Determination of adult lifespan' was also significantly enriched ($p = 0.044$, Table 1). Interestingly, one of the top GO terms was 'endoplasmic reticulum-unfolded

Table 1 Gene ontology (GO) enrichment for genes downregulated by sesamin supplementation

GO terms	*p* values
Cell projection morphogenesis	8.1×10^{-3}
Cell part morphogenesis	8.9×10^{-3}
Vulval location	1.4×10^{-2}
Neuron projection development	1.8×10^{-2}
Mating	2.2×10^{-2}
Cell morphogenesis	2.2×10^{-2}
Collagen and cuticulin-based cuticle development	2.5×10^{-2}
Response to hermaphrodite contact	2.6×10^{-2}
Neuron development	2.8×10^{-2}
Regulation of multicellular organism growth	3.2×10^{-2}
Nematode larval development	3.4×10^{-2}
Larval development	3.5×10^{-2}
Endoplasmic reticulum unfolded protein response	3.8×10^{-2}
Neuron differentiation	4.0×10^{-2}
Nonmotile primary cilium assembly	4.4×10^{-2}
Determination of adult lifespan	4.7×10^{-2}

protein response' ($p = 0.038$). Endoplasmic reticulum (ER)-unfolded protein response (UPR) genes have been shown to be negatively regulated by *sir-2.1* and to mediate (at least partially) *sir-2.1*-dependent longevity [41]. Strikingly, the expression of a subset of *abu* (activated in blocked unfolded protein response) and *pqn* (prion-like Q/N proteins) genes, both of which encode protein members of a prion-like glutamine/asparagine-rich protein family that are thought to restore protein folding in ER [42], was decreased in sesamin-fed animals (Table 2, Online Resource Table S2). The real-time PCR results confirmed that expression of the ER UPR-related *abu*/*pqn* genes were reduced by sesamin supplementation (Table 2). Based on these observations, we hypothesized that the *sir-2.1* pathway might be activated in worms that were fed sesamin.

Caenorhabditis elegans lifespan extension caused by sesamin supplementation requires *sir-2.1*

Because the microarray analysis implied the involvement of *sir-2.1*, we examined whether the longevity effect of sesamin requires *sir-2.1*. First, the lifespan of RNAi-treated worms in the presence and absence of sesamin was measured. Control RNAi-treated animals exhibited a significant increase in lifespan in the presence of sesamin, whereas *sir-2.1* RNAi-treated animals did not show a significantly different lifespan with sesamin supplementation (Fig. 1a). Second, we determined the lifespan of *sir-2.1* deletion mutants. Again, *sir-2.1* mutants did not show a significant change in lifespan with sesamin supplementation (Fig. 1b). These results strongly suggested that lifespan extension caused by sesamin requires *sir-2.1* in *C. elegans*.

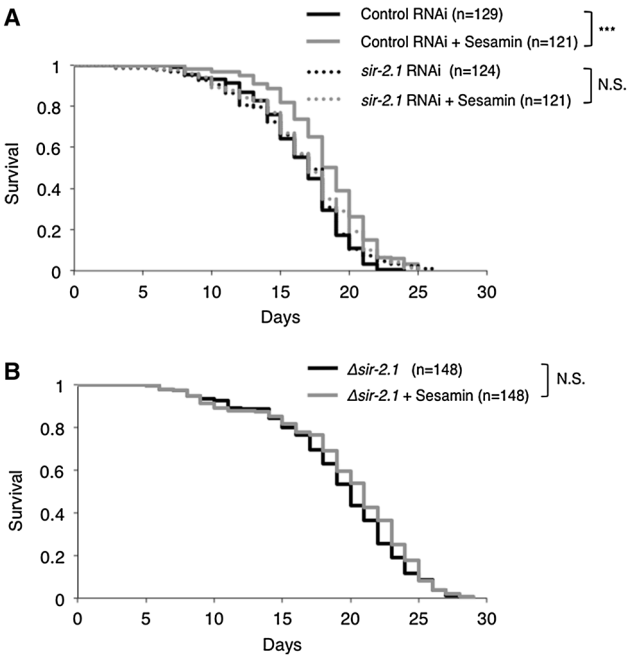

Fig. 1 Sesamin extends *C. elegans* lifespan in a *sir-2.1*-dependent manner. **a** Survival curves of RNAi-treated animals in the presence and absence of sesamin. Sesamin significantly increased lifespan in control RNAi-treated animals but failed to extend lifespan in *sir-2.1* RNAi-treated animals. Mean lifespan ± SE of control-fed (m_{cont}) and sesamin-fed (m_{sesam}) animals under the control RNAi condition was 19.9 ± 0.3 and 21.8 ± 0.3 days, respectively. m_{cont} and m_{sesam} under the *sir-2.1* RNAi condition was 19.9 ± 0.4 and 20.3 ± 0.4 days, respectively. **b** Survival curves of control-fed and sesamin-fed *sir-2.1* mutants. Loss of *sir-2.1* abolished sesamin-induced lifespan extension. m_{cont} and m_{sesam} of the *sir-2.1* mutants was 22.7 ± 0.4 and 23.2 ± 0.4 days, respectively. Survival differences were tested for significance using the log-rank test. ***$P < 0.001$. *N.S.* not significant. Detailed lifespan data and statistics are provided in Online Resource Table S3

Longevity effect of sesamin requires the autophagic gene *bec-1*

Sirtuin-mediated effects in human cell lines have been reported to be mediated (at least in part) through autophagy [43]. Morselli et al. also showed that DR and resveratrol promote longevity through the *sir-2.1*-dependent induction of autophagy in *C. elegans* [43]. To determine whether the longevity effect of sesamin is mediated by the autophagy pathway, RNAi of the *bec-1* gene, which encodes the *C. elegans* homolog of the key autophagic regulator ATG6,

Table 2 Sesamin-fed animals exhibit decreased the ER UPR-related *abu*/*pqn* gene expression

Gene	Microarray	Real-time PCR				
	Fold change	Control	±SE	Sesamin	±SE	Fold change
abu-7/*abu-8*	−2/−1.2/-1[a]	3.75	±0.88	3.01	±0.93	−0.32
abu-10	−1.4	3.39	±1.16	1.70	±0.12	−1.00
abu-13	−1.3	8.87	±3.11	3.66	±0.59	−1.28
abu-14	−1.5/−1.3[a]	16.18	±4.27	10.23	±1.55	−0.66
pqn-26	−1.9	NA	NA	NA	NA	NA
pqn-32	−2.3	5.22	±1.27	3.12	±0.44	−0.74
pqn-73	−1.1	5.63	±1.15	3.89	±0.33	−0.53

[a]Fold changes in multiple probe sets

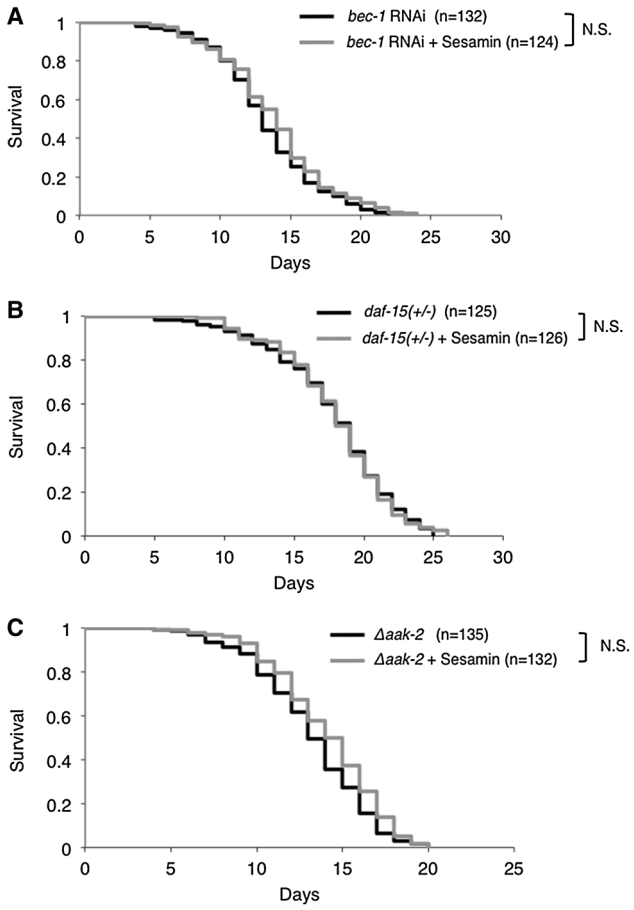

Fig. 2 Lifespan extension by sesamin supplementation requires DR-related genes. **a** Survival curves of *bec-1*/ATG6 RNAi-treated worms in the presence and absence of sesamin. m_{cont} and m_{sesam} was 16.7 ± 0.3 and 17.3 ± 0.4 days, respectively. **b** Survival curves of the *daf-15*/Raptor heterozygous mutants. m_{cont} and m_{sesam} was 21.3 ± 0.4 and 21.5 ± 0.3 days, respectively. **c** Survival curves of the *aak-2*/AMPK mutants. m_{cont} and m_{sesam} was 16.7 ± 0.3 and 17.6 ± 0.3 days, respectively. Statistical analyses were performed using the log-rank test. *N.S.* not significant. Detailed lifespan data and statistics are provided in Online Resource Table S3

was tested for an effect on lifespan extension caused by sesamin supplementation. Notably, *bec-1* RNAi-treated animals did not show a significant change in lifespan in the presence of sesamin compared with that in the absence of sesamin (Fig. 2a). These results suggested that the longevity effect of sesamin requires the autophagic gene *bec-1*.

Lifespan extension with sesamin supplementation involves the TOR pathway

DR extends lifespan, at least in part, by downregulation of the target of rapamycin (TOR) pathway [44], and inhibition of TOR triggers autophagy in yeast and mammals [45]. In addition, we found that *sinh-1*, which encodes a component of the TOR complex, was downregulated by sesamin (online resource Table S2). This observation supports the idea that sesamin might extend lifespan by inhibiting TOR. To test this possibility, we assayed mutants heterozygous for the TOR-binding partner *daf-15*/Raptor. (This test employed *daf-15* heterozygotes because *daf-15* homozygotes do not reach maturity, and because the loss of one copy of *daf-15* decreases pathway activity sufficiently to affect lifespan [46].) Lifespan extension with sesamin supplementation was suppressed in *daf-15* heterozygotes (Fig. 2b), suggesting that the longevity effect of sesamin involves the TOR pathway.

Longevity caused by sesamin supplementation requires *aak-2*/AMPK

Given that several DR-related pathways (such as the SIR-2.1 and TOR pathways) were required for lifespan extension conferred by sesamin, we suspected that the fuel sensor AMP-activated protein kinase (AMPK) might be involved. To examine the involvement of AMPK, mutants of *aak-2*, which encodes a *C. elegans* AMPK α-subunit, were assayed for lifespan extension. We found that there was no significant difference in the lifespans of *aak-2* mutants in the presence or absence of sesamin (Fig. 2c). AMPK is known to be activated upon an increase in the AMP-to-ATP ratio; we therefore compared ATP concentrations in worms grown with and without sesamin supplementation. We found no significant difference in ATP concentrations between sesamin-fed and control-fed worms (Online Resource Fig. S4). These results suggested that the longevity observed in sesamin-fed animals may be mediated by AMPK but does not involve a decrease in ATP levels.

Sesamin does not extend lifespan under DR conditions

Based on the above data, we hypothesized that sesamin might act as DR mimetic, exerting beneficial effects through a mechanism similar to that of DR. If so, sesamin supplementation should be ineffective under DR. Indeed, we found that sesamin did not prolong lifespan under DR. Specifically, under conditions of DR, sesamin-fed animals did not demonstrate a significantly longer lifespan than control animals (Fig. 3), an observation that contrasted with the increased longevity seen with sesamin supplementation when animals were given *ad libitum* access to food. These results supported the idea that sesamin mimics DR to extend lifespan in *C. elegans*. Notably, however, DR conditions significantly increased lifespan in both control-fed worms ($p < 0.001$, log-rank test) and sesamin-fed animals ($p < 0.001$, log-rank test).

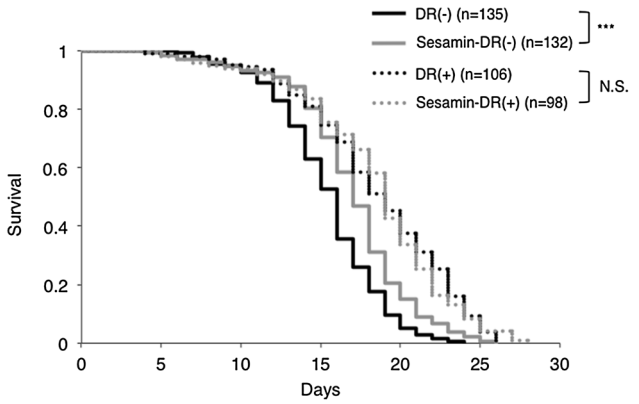

Fig. 3 Sesamin does not extend lifespan under DR conditions. Survival curves of control-fed and sesamin-fed animals under *ad libitum* and DR conditions. Sesamin supplementation and DR conditions were initiated on Day 0 and Day 2, respectively. m_{cont} and m_{sesam} under *ad libitum* conditions was 18.9 ± 0.3 and 20.4 ± 0.3 days, respectively. m_{cont} and m_{sesam} under DR conditions was 22.0 ± 0.5 and 22.0 ± 0.5 days, respectively. Although sesamin-fed animals showed a significant increase in lifespan compared with control-fed animals under *ad libitum* conditions, the two groups did not exhibit significant differences in lifespan under DR conditions. Significant differences were assessed using the log-rank test. ***$P < 0.001$. *N.S.* not significant. Detailed lifespan data and statistics are provided in Online Resource Table S3.

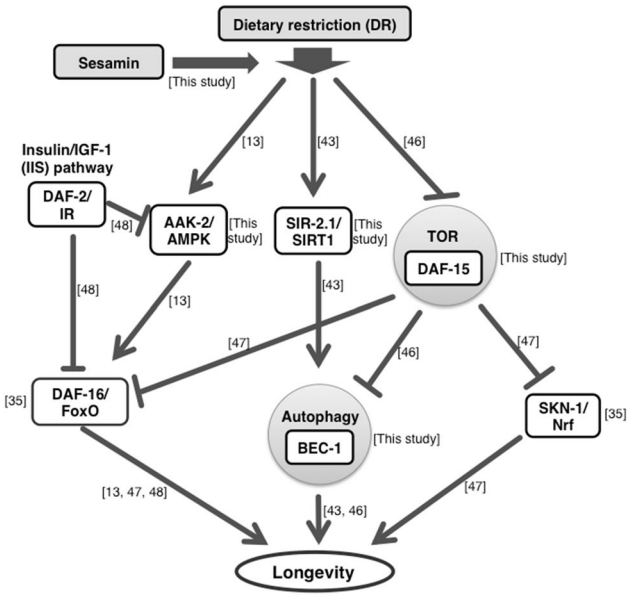

Fig. 4 Working model for the longevity effect of sesamin via DR-related nutrient-sensing pathways. This study and our previous study demonstrated that sesamin-promoted longevity requires the IIR, SIRT1/SIR2-2.1, AMPK/AAK-2, TOR-binding partner DAF-15, SKN-1/Nrf, and an autophagic protein BEC-1. Because all of these proteins or pathways have been reported to mediate a DR signal, we propose a working model in which sesamin might extend lifespan by acting upstream of the DR-associated nutrient-sensing pathways. References are indicated in *square brackets*. Inductive pathways are shown as *arrows*, and suppressive pathways are shown as *arrows* with *T-shaped heads*

This observation suggested that sesamin supplementation is unlikely to prolong lifespan beyond that obtained with DR.

Discussion

The longevity response to DR is actively regulated by nutrient-sensing pathways involving sirtuins, TOR, AMPK, and insulin/IGF-1 (IIS) signaling and are conserved from yeast to mammals [14]. In this study, we showed that sesamin increases the lifespan of the nematode *C. elegans* through the *sir-2.1*/SIRT1, TOR, and *aak-2*/AMPK pathways. In addition, we have previously reported that sesamin-associated lifespan extension depended on the insulin/IGF-1 (IIS) pathway [35]. Therefore, lifespan extension by sesamin is likely to involve nearly every known DR-related nutrient-sensing pathways. Based on this evidence, we propose a working model, in which sesamin might extend lifespan by acting upstream of the aforementioned nutrient-sensing pathways (Fig. 4). DR-triggered SIR-2.1 activation induces autophagy and induction of autophagy promotes longevity in *C. elegans* [43]. DR also activates AAK-2/AMPK such that AMPK activates the DAF-16/FoxO transcription factor [13]. At the same time, DR suppresses the TOR pathway, resulting in the induction of autophagy [46] and the activation of DAF-16/FoxO [47]. SKN-1/Nrf also mediates the TOR-dependent longevity [47]. The IIS signaling pathway regulates DR-induced longevity through the suppression of DAF-16/FoxO and AAK-2/AMPK [48]. We have previously reported that the sesamin-associated extension of *C. elegans* lifespan depended on the DAF-16/FoxO and SKN-1/Nrf transcription factors [35]. The present study showed that lifespan extension by sesamin supplementation depends on SIR-2.1/SIRT1, a TOR component (DAF-15), AAK-2/AMPK, and a key regulator of autophagy (BEC-1). Considered together, sesamin might mimic a DR signal to promote longevity in *C. elegans*.

It is unlikely that sesamin exerts its effects by causing DR-like decreases in body weight and fertility. Notably, we have previously demonstrated that nematode growth curves and the numbers of offspring were unaffected by sesamin supplementation [35]. Nevertheless, sesamin extends the lifespan of *C. elegans* through signaling pathways also employed by DR. These features are reminiscent of those of resveratrol, a candidate DR mimetic, which exerts beneficial aspects of DR without involving trade-off effects, such as decreased fertility, due to reduced calorie intake [25].

Well-known candidate DR mimetic compounds include resveratrol [49], 2-deoxyglucose (2DG) [50],

and metformin [51]. Resveratrol has been reported to act through *sir-2.1*/SIRT [25, 41] and *aak-2*/AMPK [13]. Metformin has been proposed to extend lifespan in *C. elegans* and mice by activating AMPK [19, 52]. 2DG has been shown to act via *aak-2*/AMPK, but not via *sir-2.1*/SIRT1, in *C. elegans* [53]. The combination of our previous work and the current study shows that sesamin extends *C. elegans* lifespan through the *sir-2.1*/SIRT1, *aak-2*/AMPK, TOR, and IIS pathways. These results suggest that sesamin may act, at least in part, through mechanisms that are non-overlapping with known candidate DR mimetics.

It is important to mention that the role of Sir2 in CR-mediated lifespan extension has remained contentious, especially in yeast [11]. The initially proposed role of Sir2 was based on the finding that short-lived strains lacking Sir2 did not have extended lifespan under CR conditions [9]. However, the finding that CR fails to increase life span in a strain lacking Sir2 can be interpreted in two ways: (1) CR directly increases Sir2 activity or (2) yeast strains lacking Sir2, which have an approximately 50% reduction in mean replicative life-span potential, do not live long enough to respond to CR [11]. Several studies have shown that Sir2-independent CR-mediated lifespan extension in yeast [54, 55]. As is the case for *C. elegans*, lifespan extension by DR is either abrogated or not affected by *sir-2.1* deletion, depending on the chosen method of DR [11]. Although it is undisputed that *sir-2.1* is one of the mediators for DR-associated longevity [11, 14], the data should be interpreted with caution and follow-up studies will be needed to confirm the mechanism underlying *sir-2.1*-dependent lifespan extension by sesamin.

Sesamin's direct target remains unclear, but several known pathways or proteins have been implicated in mediating the effects of sesamin. For instance, sesamin metabolites have been shown to activate the Nrf2/ARE (antioxidant response element) signaling pathway in rat pheochromocytoma PC12 cells [56]. Sesamin has also been shown to induce autophagy in colon cancer cells by reducing the tyrosine phosphorylation of EphA1 and EphB2 [57]. It is critical to identify the target(s) of sesamin and to examine potential longevity effects of sesamin in mammals, including primates and humans.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Fontana L, Partridge L, Longo VD (2010) Extending healthy life span–from yeast to humans. Science 328(5976):321–326. doi:10.1126/science.1172539
2. McCay C, Crowell M, Maynard L (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr 10:63–79
3. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325(5937):201–204. doi:10.1126/science.1173635
4. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun 5:3557. doi:10.1038/ncomms4557
5. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, Longo DL, Allison DB, Young JE, Bryant M, Barnard D, Ward WF, Qi W, Ingram DK, de Cabo R (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 489(7415):318–321. doi:10.1038/nature11432
6. Partridge L, Piper MD, Mair W (2005) Dietary restriction in Drosophila. Mech Ageing Dev 126(9):938–950. doi:10.1016/j.mad.2005.03.023
7. Klass MR (1977) Aging in the nematode Caenorhabditis elegans: major biological and environmental factors influencing life span. Mech Ageing Dev 6(6):413–429
8. Lakowski B, Hekimi S (1998) The genetics of caloric restriction in Caenorhabditis elegans. Proc Natl Acad Sci USA 95(22):13091–13096
9. Lin SJ, Defossez PA, Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 289(5487):2126–2128
10. Imai S, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403(6771):795–800. doi:10.1038/35001622
11. Longo VD, Kennedy BK (2006) Sirtuins in aging and age-related disease. Cell 126(2):257–268. doi:10.1016/j.cell.2006.07.002
12. Wang Y, Tissenbaum HA (2006) Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. Mech Ageing Dev 127(1):48–56. doi:10.1016/j.mad.2005.09.005
13. Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A (2007) An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr Biol 17(19):1646–1656. doi:10.1016/j.cub.2007.08.047
14. Kenyon CJ (2010) The genetics of ageing. Nature 464(7288):504–512. doi:10.1038/nature08980
15. Rogina B, Helfand SL (2004) Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci USA 101(45):15998–16003. doi:10.1073/pnas.0404184101
16. Guarente L (2013) Calorie restriction and sirtuins revisited. Genes Dev 27(19):2072–2085. doi:10.1101/gad.227439.113

17. Chen D, Steele AD, Lindquist S, Guarente L (2005) Increase in activity during calorie restriction requires Sirt1. Science 310(5754):1641. doi:10.1126/science.1118357
18. Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, Estey C, Moffat C, Crawford S, Saliba S, Jardine K, Xuan J, Evans M, Harper ME, McBurney MW (2008) SirT1 regulates energy metabolism and response to caloric restriction in mice. PLoS One 3(3):e1759. doi:10.1371/journal.pone.0001759
19. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4:2192. doi:10.1038/ncomms3192
20. Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305(5682):390–392. doi:10.1126/science.1099196
21. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, Kim J, Yancopoulos G, Valenzuela D, Murphy A, Yang Y, Chen Y, Hirschey MD, Bronson RT, Haigis M, Guarente LP, Farese RV, Weissman S, Verdin E, Schwer B (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Mol Cell Biol 27(24):8807–8814. doi:10.1128/MCB.01636-07
22. Nakagawa T, Guarente L (2009) Urea cycle regulation by mitochondrial sirtuin, SIRT5. Aging 1(6):578–581. doi:10.18632/aging.100062
23. Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, Smith SR, Ravussin E, Team CP (2007) Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS Med 4(3):e76. doi:10.1371/journal.pmed.0040076
24. Baur JA (2010) Resveratrol, sirtuins, and the promise of a DR mimetic. Mech Ageing Dev 131(4):261–269. doi:10.1016/j.mad.2010.02.007
25. Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430(7000):686–689. doi:10.1038/nature02789
26. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444(7117):337–342. doi:10.1038/nature05354
27. Marchal J, Pifferi F, Aujard F (2013) Resveratrol in mammals: effects on aging biomarkers, age-related diseases, and life span. Ann N Y Acad Sci 1290:67–73. doi:10.1111/nyas.12214
28. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8(2):157–168. doi:10.1016/j.cmet.2008.06.011
29. Sirato-Yasumoto S, Katsuta M, Okuyama Y, Takahashi Y, Ide T (2001) Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. J Agric Food Chem 49(5):2647–2651
30. Gu JY, Wakizono Y, Tsujita A, Lim BO, Nonaka M, Yamada K, Sugano M (1995) Effects of sesamin and alpha-tocopherol, individually or in combination, on the polyunsaturated fatty acid metabolism, chemical mediator production, and immunoglobulin levels in Sprague-Dawley rats. Biosci Biotechnol Biochem 59(12):2198–2202
31. Hirose N, Doi F, Ueki T, Akazawa K, Chijiiwa K, Sugano M, Akimoto K, Shimizu S, Yamada H (1992) Suppressive effect of sesamin against 7,12-dimethylbenz[a]-anthracene induced rat mammary carcinogenesis. Anticancer Res 12(4):1259–1265
32. Matsumura Y, Kita S, Tanida Y, Taguchi Y, Morimoto S, Akimoto K, Tanaka T (1998) Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats. Biol Pharm Bull 21(5):469–473
33. Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H (1996) Hypocholesterolemic effect of sesame lignan in humans. Atherosclerosis 122(1):135–136
34. Ide T, Ashakumary L, Takahashi Y, Kushiro M, Fukuda N, Sugano M (2001) Sesamin, a sesame lignan, decreases fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1. Biochim Biophys Acta 1534(1):1–13
35. Yaguchi Y, Komura T, Kashima N, Tamura M, Kage-Nakadai E, Saeki S, Terao K, Nishikawa Y (2014) Influence of oral supplementation with sesamin on longevity of *Caenorhabditis elegans* and the host defense. Eur J Nutr 53(8):1659–1668. doi:10.1007/s00394-014-0671-6
36. Finch CE, Ruvkun G (2001) The genetics of aging. Annu Rev Genom Hum Genet 2:435–462. doi:10.1146/annurev.genom.2.1.435
37. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77(1):71–94
38. Kashima N, Fujikura Y, Komura T, Fujiwara S, Sakamoto M, Terao K, Nishikawa Y (2012) Development of a method for oral administration of hydrophobic substances to Caenorhabditis elegans: pro-longevity effects of oral supplementation with lipid-soluble antioxidants. Biogerontology 13(3):337–344. doi:10.1007/s10522-012-9378-3
39. Wu D, Rea SL, Yashin AI, Johnson TE (2006) Visualizing hidden heterogeneity in isogenic populations of C. elegans. Exp Gerontol 41(3):261–270. doi:10.1016/j.exger.2006.01.003
40. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biol 2(1):RESEARCH0002. doi:10.1186/gb-2000-2-1-research0002
41. Viswanathan M, Kim SK, Berdichevsky A, Guarente L (2005) A role for SIR-2.1 regulation of ER stress response genes in determining *C. elegans* life span. Dev Cell 9(5):605–615. doi:10.1016/j.devcel.2005.09.017
42. Urano F, Calfon M, Yoneda T, Yun C, Kiraly M, Clark SG, Ron D (2002) A survival pathway for *Caenorhabditis elegans* with a blocked unfolded protein response. J Cell Biol 158(4):639–646. doi:10.1083/jcb.200203086
43. Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, Criollo A, Galluzzi L, Malik SA, Vitale I, Michaud M, Madeo F, Tavernarakis N, Kroemer G (2010) The life span-prolonging effect of sirtuin-1 is mediated by autophagy. Autophagy 6(1):186–188
44. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C (2008) A role for autophagy in the extension of lifespan by dietary restriction in C. elegans. PLoS Genet 4(2):e24. doi:10.1371/journal.pgen.0040024

45. Wullschleger S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. Cell 124(3):471–484. doi:10.1016/j.cell.2006.01.016
46. Jia K, Chen D, Riddle DL (2004) The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development 131(16):3897–3906. doi:10.1242/dev.01255
47. Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, Sabatini DM, Blackwell TK (2012) TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab 15(5):713–724. doi:10.1016/j.cmet.2012.04.007
48. Apfeld J, O'Connor G, McDonagh T, DiStefano PS, Curtis R (2004) The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. Genes Dev 18(24):3004–3009. doi:10.1101/gad.1255404
49. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425(6954):191–196. doi:10.1038/nature01960
50. Roth GS, Ingram DK, Lane MA (2001) Caloric restriction in primates and relevance to humans. Ann N Y Acad Sci 928:305–315
51. Dhahbi JM, Mote PL, Fahy GM, Spindler SR (2005) Identification of potential caloric restriction mimetics by microarray profiling. Physiol Genom 23(3):343–350. doi:10.1152/physiolgenomics.00069.2005
52. Onken B, Driscoll M (2010) Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS One 5(1):e8758. doi:10.1371/journal.pone.0008758
53. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6(4):280–293. doi:10.1016/j.cmet.2007.08.011
54. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol 2(9):E296. doi:10.1371/journal.pbio.0020296
55. Kennedy BK, Smith ED, Kaeberlein M (2005) The enigmatic role of Sir2 in aging. Cell 123(4):548–550. doi:10.1016/j.cell.2005.11.002
56. Hamada N, Tanaka A, Fujita Y, Itoh T, Ono Y, Kitagawa Y, Tomimori N, Kiso Y, Akao Y, Nozawa Y, Ito M (2011) Involvement of heme oxygenase-1 induction via Nrf2/ARE activation in protection against H2O2-induced PC12 cell death by a metabolite of sesamin contained in sesame seeds. Bioorg Med Chem 19(6):1959–1965. doi:10.1016/j.bmc.2011.01.059
57. Tanabe H, Kuribayashi K, Tsuji N, Tanaka M, Kobayashi D, Watanabe N (2011) Sesamin induces autophagy in colon cancer cells by reducing tyrosine phosphorylation of EphA1 and EphB2. Int J Oncol 39(1):33–40. doi:10.3892/ijo.2011.1011