Methionine Sulfoxide Reductase A Mediates Dietary Restriction-Induced Lifespan Extension in Caenorhabditis elegans

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Introduction

Dietary restriction (DR) is defined as a decrease in the nutrient uptake of an organism without causing malnutrition. DR is the most consistent and reproducible method used to increase lifespan and has been shown to do so in over twenty different species [1-3]. Although the effect of DR on life span has been extensively studied the exact mechanism by which DR acts is still not fully understood. However, there is considerable evidence that the lifespan extension seen in DR is due to a reduction in oxidative damage [4]. The modified free radical theory of aging proposed that reactive oxygen species (ROS) derived from oxygen are responsible for cellular damage associated with aging. This could be due to a decrease in the generation of reactive oxygen species (ROS) and/or an increase in the cellular protective mechanisms against oxidative damage under conditions of DR. As example, rodents subjected to DR showed a decrease in the age-associated production of mitochondrial ROS and slower accumulation of oxidative damage [4]. In addition, there are several reports of life span extension, independent of DR, that have resulted from over-expression of enzymes such as superoxide dismutase (SOD) [5], catalase [6] and MsrA [7], which are known to protect cells against oxidative damage either by destroying ROS or, as in the case of MsrA, repairing damage to proteins due to methionine oxidation [8]. The MSR system is unique in that it can also function as part of an ROS scavenger system in which methionine residues in proteins can function as catalytic antioxidants [9].

The methionine sulfoxide reductase system is a highly conserved system [10]. Methionine residues are very susceptible to oxidation by ROS and are converted to either the S epimer of methionine sulfoxide (Met-S(o)) or the R-epimer (Met-R(o)) when chemically oxidized by ROS. The methionine oxidation can be reversed by the methionine sulfoxide reductase system, MsrA and MsrB [8,10]. MsrA reduces the S epimer of Met(o) in proteins and MsrB is specific for the R epimer of Met(o) [11-15].

Several studies have provided evidence that MsrA is important in protecting cells against oxidative damage and in the aging process. Escherichia coli and yeast mutants lacking MsrA have an increased sensitivity to oxidative stress [16-18]. Compared to wild type mice, MsrA-/- mice also show decreased resistance to oxidative stress [19,20]. Moreover, expression of both MsrA and MsrB declines in senescent human fibroblasts cells compared to young cells, and this decline is associated with accumulation of oxidized proteins [21]. Interestingly, over-expression of MsrA lowers the levels of ROS in cells [22] and increases lifespan in fruit flies and yeast [7,23]. In C. elegans, axenic medium imposes dietary restriction (ADR) and extends C. elegans lifespan [24]. ADR was found to cause higher activities of the antioxidant enzymes SOD and catalase [24]. C. elegans fed with a dilution of bacteria in liquid medium (bacterial dietary restriction: BDR) showed an increased lifespan, which was dependent on PHA-4, a FOXA transcription factor [25]. Interestingly, PHA-4 was found to mediate BDR-induced longevity by upregulating SOD [25]. Recently, a C. elegans homolog of MsrA has been identified [26] and an msra deletion mutant was reported to be sensitive to oxidative stress [27]. Moreover, this deletion mutation of msra decreases the lifespan of the long-lived daf-2 mutant worms [27]. Interestingly, the DAF-16/Foxo3 transcription factor, which is a major target of the daf-2

Keywords: MsrA; Dietary restriction; Aging; Lifespan; C. elegans

Abstract

Background: Methionine sulfoxide reductase A (MsrA) is a well-studied antioxidant enzyme that has been found to be important for protecting cells against oxidative damage and regulating lifespan in several species. However, the role of MsrA in dietary restriction has not been examined. The authors evaluated the function of MsrA in dietary restriction-induced lifespan extension in Caenorhabditis elegans.

Methods: C. elegans loss-of-function msra mutant animals and wild type control animals were subjected to two widely used dietary restriction treatments, solid dietary restriction (sDR) and dietary restriction by liquid bacteria (BDR). The survival of the animals was evaluated and the data was statistically analyzed.

Results: The loss-of-function mutation of msra significantly suppressed the lifespan extension conferred by solid dietary restriction. By contrast, msra was dispensable for lifespan extension resulting from dietary restriction by diluted bacteria in liquid.

Conclusion: msra-1 is a major factor in the sDR-induced lifespan extension. This result, coupled with the previous finding that MsrA mediates the effect of insulin-like signaling on lifespan extension, indicates an essential role of MsrA in the aging process in C. elegans.

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insulin-like signaling pathway, positively regulates msra expression [27]. However, the role of MsRA in DR has not been examined. Here we show that MsRA mediates the effect of solid dietary restriction on lifespan extension in C. elegans.

Materials and Methods

C. elegans strains

The Bristol N2 strain is the wild type C. elegans strain used in all experiments. Strains were maintained at 20°C as described by Brenner [28]. To obtain the homozygous msra-1(tm1421) mutant, N2 males were crossed to the msra-1(tm1421) II; lin-15B(n765ts) mutants. The outcross progeny were selected and self-propagated to remove the lin-15B(n765ts) mutation. Fifty msra-1 mutant worms were examined for the homozygous deletion mutation by following a standard single-worm PCR [29] and all tested worms showed the homozygous deletion. The sequences of the primers used were:

- msra-1 F: 5’ - CACATTGGATTCGCCCCGATT - 3’;
- msra-1 R: 5’ - GACAACACTCTTCAATCCATT - 3’.

Solid dietary restriction (sDR) lifespan analysis

The sDR method was modified from that previously described [30]. Briefly, the OP50 overnight culture was washed and re-suspended in S Basal media without cholesterol. Serial dilutions were performed to achieve bacterial concentrations of 5.0×10^11, 5.0×10^10, 5.0×10^9, and 5.0×10^8 bacteria/ml. 250 µl of these diluted bacterial cultures were spotted on each 60 mm plate on the day of transfer. 30 adult worms were placed on each plate containing various concentrations of bacteria starting at day 1 of adulthood. Three plates for each strain at each bacterial concentration were set up. In the first week of the lifespan experiments, DR plates that contain FUdR (20 µg/ml) were used to prevent progeny from hatching. Worms were transferred to fresh plates every other day. Dietary restriction (sDR) was considered such as decreasing food availability by bacteria dilution in liquid culture (BDR) [31] and on solid medium (sDR) [30]. Since DAF-16 is required for sDR-mediated lifespan extension [30] and msra-1 is a target gene of DAF-16 [27], we initially examined the role of msra-1 in plate-based dietary restriction (sDR). Consistent with a previous report [30], the lifespan of N2 worms fed with 5×10^10 bacteria/ml (DR) is significantly increased compared to that of animals fed with 5×10^10 bacteria/ml (AL: ad libitum). The mean lifespan is increased by 38% (from 21.25 days to 28.88 days; P<0.0001, log-rank test) and the maximum lifespan is extended by 20 days (from 31 to 51 days) (Figure 1 and Table 1). The sDR-treated msra-1 mutants have a slightly shorter (but significant) median life span than sDR-treated N2 animals (26.13 days vs. 28.88 days, P=0.0002). However, the most striking effect seen with the msra-1 mutants was on the maximum lifespan. The maximum lifespan of sDR-treated msra-1 mutants is 15 days shorter than that of N2 worms (36 vs. 51 days) (Figure 1 and Table 1). It should be noted that the mean lifespan of sDR-treated msra-1 mutants is still increased

Statistical Analysis

GraphPad Prism 5 was used to generate survival curves and determine medians, means and percentiles. In all cases, P-values were calculated using the log-rank (Mantel–Cox) method.

Results and Discussion

Previous studies have shown that ADR and BDR extend C. elegans lifespan and up-regulate antioxidant enzymes [24]. PHA-4 (FOXA) was reported to mediate BDR-induced longevity by controlling SOD transcription [25]. Our goal was to determine whether MsRA may also be required for DR-mediated lifespan extension. A C. elegans msra-1(tm1421) deletion mutant has been isolated [27], and we initially confirmed the homozygous deletion in the msra-1 mutant. Fifty msra-1 mutant worms were analyzed by using single worm PCR with primers flanking the deletion site. As expected, a 171 bp deletion band was detected in all msra-1(tm1421) mutant worms, which confirmed the 908 bp deletion in the msra-1(tm1421) mutant (Figure S1).

Next, the msra-1(tm1421) mutant worms were subjected to dietary restriction to examine the possible role of msra-1 in DR-mediated lifespan extension. Various dietary restriction protocols have been developed in C. elegans such as decreasing food availability by bacterial dilution in liquid culture (BDR) [31] and on solid medium (sDR) [30]. Since DAF-16 is required for sDR-mediated lifespan extension [30] and msra-1 is a target gene of DAF-16 [27], we initially examined the role of msra-1 in plate-based dietary restriction (sDR). Consistent with a previous report [30], the lifespan of N2 worms fed with 5×10^10 bacteria/ml (DR) is significantly increased compared to that of animals fed with 5×10^10 bacteria/ml (AL: ad libitum). The mean lifespan is increased by 38% (from 21.25 days to 28.88 days; P<0.0001, log-rank test) and the maximum lifespan is extended by 20 days (from 31 to 51 days) (Figure 1 and Table 1). The sDR-treated msra-1 mutants have a slightly shorter (but significant) median life span than sDR-treated N2 animals (26.13 days vs. 28.88 days, P=0.0002). However, the most striking effect seen with the msra-1 mutants was on the maximum lifespan. The maximum lifespan of sDR-treated msra-1 mutants is 15 days shorter than that of N2 worms (36 vs. 51 days) (Figure 1 and Table 1). It should be noted that the mean lifespan of sDR-treated msra-1 mutants is still increased.

![Figure 1: MSRA-1 mediates sDR-induced lifespan extension. Lifespan curves of wild-type N2 animals and msra-1 mutant worms treated by sDR (5×10^10 bacteria/ml) and fed ad libitum (5×10^10 bacteria/ml). N2 + AL, mean lifespan 28.88 days and maximum 51 days; N2 + sDR, mean lifespan 28.88 days and maximum 51 days; msra-1 + AL, mean 21.25 days and maximum 31 days; msra-1 + sDR, mean 26.13 days and maximum 36 days. This entire experiment was done twice and similar results were obtained. The pooled data from these two experiments are shown in this figure.](image-url)
by 5 days compared to that of msra-1 mutant worms fed ad libitum (from 21.38 days to 26.13 days, P<0.0001), suggesting that the msra-1 mutation did not completely suppress the effect of sDR on the mean lifespan. As shown in Table 1 and Figure 1 the N2 and msra-1 mutants have a similar lifespan when fed ad libitum (mean life spans are 21.25 and 21.38 days, respectively, and the maximum lifespans are 31 and 32 days, respectively). This is in contrast to a previous study showing that mutant worms lived 30% shorter than N2 [27] when fed ad libitum (mean life spans are 21.38 days to 26.13 days, P<0.0001). This suggests that the mutation did not completely suppress the effect of sDR on the mean lifespan. The results indicate that the optimal bacterial concentration to maximize lifespan extension for msra-1 mutants is 1.5×10⁸ cells/ml, but it is 7.5×10⁷ for N2 animals (Figure 2 and Table 1). Previous published studies have indicated that C. elegans with different genotypes can respond to DR differentially. Therefore, the concentration of bacteria in the food that maximizes lifespan for one genotype may be different from the one works for another genotype [32].

In C. elegans, dietary restriction has been mainly applied in four ways: dilution of bacteria in liquid medium (BDR) [31], eat-2 mutants with pharyngeal defect and insufficient food intake [33], culture in axenic medium (ADR) [24] and dilution of bacteria on solid medium plate (sDR) [30]. All of these protocols extend C. elegans lifespan. It is of interest, and unexpected, that different forms of DR extend lifespan by different mechanisms [3,32,34]. For example, PHA-4, a FOXA transcription factor, is required for BDR-induced lifespan extension and for the longevity phenotype of eat-2 mutants [25]. In contrast, although DAF-16, a different FOXO transcription factor, is dispensable for BDR-mediated lifespan extension, it is essential for sDR treatment to increase C. elegans lifespan [30]. It was suggested that different dietary restriction protocols may require different effectors for lifespan extension, possibly due to the fact that some nutrients may be more

| Strain and culture conditions | Mean lifespan (days) | Max. lifespan (days) | N¹ | P Value² |
|-------------------------------|---------------------|---------------------|----|----------|
| sDR                           |                     |                     |    |          |
| N2 + 5×10⁸ cells/ml (AL)       | 21.25 ± 0.6291      | 31                  | 145(31) |          |
| N2 + 5×10⁸ cells/ml (DR)       | 28.88 ± 1.0078      | 51                  | 63(44) | < 0.0001 (vs. N2 + AL) |
| msra-1+ 5×10⁸ cells/ml (AL)    | 21.38 ± 0.9885      | 32                  | 142(29) | 0.2083 (vs. N2 + AL) |
| msra-1+ 5×10⁸ cells/ml (DR)    | 26.13 ± 1.3901      | 36                  | 72(24) | 0.0002 (vs. N2 + DR) |
| BDR                           |                     |                     |    |          |
| N2 + 7.5×10⁶ cells/ml(AL)      | 34.25 ± 3.7666      | 50                  | 55(5) |          |
| N2 + 7.5×10⁷ cells/ml (DR)     | 42.5 ± 1.8027       | 55                  | 49(11) | 0.0005 (vs. N2 + AL) |
| msra-1+ 7.5×10⁸ cells/ml(AL)   | 38.5 ± 1.6583       | 48                  | 54(6) |          |
| msra-1+ 1.5×10⁹ cells/ml(DR)   | 44.45 ± 1.5562      | 60                  | 49(11) | < 0.0001 (vs. msra-1 + AL) |

¹N = population size, numbers of censored animals are indicated in parenthesis;
²P values (log-rank test) for mean life span of each group compared to group indicated in parentheses.
limiting than others depending on the DR method [34]. For example, sDR might reduce carbohydrates more severely than amino acids. Thus, sDR is dependent on FOXO that regulates carbohydrates metabolism. By contrast, another DR method BDR may mainly reduce amino acids. Therefore, BDR requires the Foxa/pha-4 transcription factor that has recently been found to be downstream of TOR (target-of-rapamycin) [35], the well-known amino-acid responsive pathway [36]. Interestingly, both PHA-4 and DAF-16 regulate transcription of the antioxidant enzyme superoxide dismutase [25]. BDR involves the PHA-4-dependent expression of sod-1, sod-2 and sod-5. DAF-16 regulates the expression of sod-1, sod-3 and sod-5 in response to reduced daf-2 insulin-like signaling [25].

Similar to SOD, expression of msra-1 is also positively regulated by DAF-16 [27]. When the daf-2 insulin-like signal is reduced or when C. elegans is under oxidative stress, the expression of msra-1 is up-regulated dependent on DAF-16 [27]. The lifespan results presented here suggest that msra-1 is a major factor in the sDR-induced lifespan extension because it is a target gene of DAF-16 (Figure 3), since both DAF-16 and Msra are required for sDR, but not BDR, to extend C. elegans lifespan. Interestingly, dietary restriction was found to alleviate abnormal locomotor activity and dopamine levels of Msra-/- mice, suggesting that DR can prevent some of the oxidative damage seen in Msra-/- mice [37], although in another study using Msra-/- mice there was no evidence of abnormal locomotor activity [20]. In addition, the influence of Msra on yeast lifespan was independent of DR (growth in the presence of 0.5% glucose) [23]. Recently, it was reported that yeast forkhead box transcription factors FKH1 and FKH2, putative DAF-16 orthologs, are required for lifespan extension induced by severe caloric restriction (SCR) in which yeast cells are maintained in water [38]. However, the role of Msra in these studies was not evaluated. The above results suggest different dietary restriction protocols may require different effectors for lifespan extension, as observed in C. elegans.

Since the mean lifespan of the sDR-treated msra-1 mutant increased slightly, it appears that other daf-16 target genes may also be required to achieve the full mean lifespan extension by sDR. It has been previously shown that msra-1 accounts for most of the longevity of daf-2 mutants, and msra-1 is certainly the major factor involved in the maximum lifespan extension in the present studies using sDR. Thus, msra-1, as a target gene of the DAF-16 FOXO transcription factor, is essential for life span extension conferred by both dietary restriction and reduction of the daf-2 insulin-like signaling pathway (Figure 3).

Conclusions

We provide genetic evidence that methionine sulfoxide reductase A (Msra) plays an essential role in dietary restriction-mediated (sDR) lifespan extension in C. elegans. Msra is an evolutionarily conserved antioxidant enzyme that has been found to be important for protecting cells against oxidative damage and regulating lifespan in several species. Interestingly, human Foxo3 positively regulates the expression of human Msra and activates the C. elegans msra-1 promoter in human HEK293 cells [27]. This suggests msra-1 may be required for the health-beneficial effect of dietary restriction in other species including humans.

Summary

Dietary restriction has been shown to increase the lifespan of many species varying from yeast to rodents. Methionine sulfoxide reductase A (Msra) is a well-studied antioxidant enzyme that has been found to be important for protecting cells against oxidative damage and regulating lifespan in several species. However, the role of Msra in dietary restriction has not been examined. Recently, an ortholog of Msra (Msra-1) has been identified in Caenorhabditis elegans and it is required for the longevity phenotype of a mutant with reduced insulin-like signaling. Here we show that a loss-of-function mutation of msra-1 significantly suppresses the lifespan extension conferred by solid dietary restriction (sDR) in C. elegans. We also found that Msra, like its positive regulator DAF-16/FOXO transcription factor, is dispensable for lifespan extension resulted from dietary restriction by diluted bacteria in liquid. These data suggest msra-1 is a major factor in the sDR-induced lifespan extension because it is a target gene of DAF-16. This result, coupled with the previous finding that Msra mediates the effect of insulin-like signaling on lifespan extension, indicates an essential role of Msra in the aging process in C. elegans. Interestingly, human FOXO3a has been shown to regulate expression of msra and can bind to the C. elegans msra-1 promoter, suggesting Msra may be required for the health-beneficial effect of dietary restriction in other species including humans.

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