The Role of the Presenilin-1 Homologue Gene sel-12 of Caenorhabditis elegans in Apoptotic Activities*

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Many cases of autosomal dominant early onset familial Alzheimer’s disease result from mutations in presenilin-1 (PS1). In this study, we examined the role of the PS1 homologue gene sel-12 of Caenorhabditis elegans under oxidative stress and clarified the sel-12-induced apoptosis. A genetic null allele mutant, sel-12(ar171), showed resistance to oxidative stress and prevented mitochrondrial dysfunction-induced apoptosis. On the other hand, another allele mutant, sel-12(ar131), that carries a missence mutation showed a proapoptotic activity, which may be the result of a gain of function property. Also, sel-12(ar131)-induced apoptosis was sel-12(ar131)-dependent. Dantrolene, which specifically inhibits Ca2+ release from endoplasmic reticulum stores, prevents sel-12(ar131)-induced apoptosis. SEL-12, which is localized in the endoplasmic reticulum, may induce apoptosis through abnormal calcium release from the endoplasmic reticulum. Together, with the previous finding that human PS1 could substitute for SEL-12, these results suggest the similar involvement of PS1-inducing apoptosis under oxidative stress and mitochondrial dysfunction in the Alzheimer’s Disease brain.

Many cases of autosomal dominant early onset familial Alzheimer’s disease (FAD) result from mutations in the gene encoding presenilin-1 (PS1) (1). PS1 is an integral membrane protein comprising multiple transmembrane domains that is expressed in neurons throughout the brain and primarily localized in the endoplasmic reticulum (ER) and the Golgi apparatus (2, 3). PS1 is essential for γ-secretase cleavage of the amyloid precursor protein (APP) and Notch protein (4–7). FAD-related mutations in PS1 increase the levels of Aβ42 in Alzheimer’s disease brains (8), transfected cell lines, and transgenic animals (9, 10). It is recognized that oxidative stress and mitochondrial abnormalities may play important roles in the pathogenesis of Alzheimer’s disease (11, 12). Overexpression of PS1 mutations in cultured cells increases their vulnerability to oxidative stress and Aβ toxicity (13, 14). As it is well known that oxidative stress plays the role of apoptosis (15), the oxidative stress and apoptosis may be also tightly linked in Alzheimer’s disease. In fact, FAD-related mutant PS1 is also involved in apoptosis (13, 16).

Caenorhabditis elegans has the PS1 homologue gene sel-12, which facilitates lin-12/Notch receptor-mediated signaling, and the mutation of sel-12 produces defects in vulva and neuronal development (17, 18). Leivitan et al. (19) have shown that human PS1 could substitute for C. elegans sel-12 protein. In this study, we try to elucidate the possibility that sel-12 is involved in oxidative stress and apoptosis in C. elegans. In attempting to clarify the relationship between sel-12 and cell death, we used the C. elegans sel-12 and mev-1 mutants in this study. The mev-1(kn1) mutant has a defect in the cytochrome b large subunit in complex II of the mitochondrial electron transport chain that shows hypersensitivity to oxygen and paraquat (20).

Senoo-Matsuda et al. (21) have reported that the generation of reactive oxygen species is elevated in mev-1 mutants. Furthermore, increased numbers of apoptosis were observed in mev-1(kn1) embryos. For these reasons, we chose to investigate sel-12/mev-1 double mutants in this study. The sel-12 mutants examined in this study were sel-12(ar131)unc-1(e538) and sel-12(ar171)unc-1(e538). ar131 carries a missence mutation (C60S), and ar171 carries a nonsense mutation (W225stop), which produces a truncated SEL-12 protein. Genetically, sel-12(ar171) represents a null allele (17). The marker mutant unc-1(e538) was also examined as a control. Moreover, we demonstrate in this study that intracellular calcium regulation is an important factor for sel-12-induced apoptosis by the use of dantrolene to block calcium release from the ER.

EXPERIMENTAL PROCEDURES

Nematode Propagation—The C. elegans alleles used were as follows: Bristol N2 wild-type strain, unc-1(e538); sel-12(ar131)unc-1(e538); sel-12(ar171)unc-1(e538); ced-3(n1286); ced-3(n1286)sel-12(ar131)unc-1(e538); ced-3(n1894); ced-4(n1894)sel-12(ar131)unc-1(e538); mev-1(kn1); mev-1(kn1)lon-1(e185); and sel-12(ar171)unc-1(e538)mev-1(kn1)lon-1(e185). The N2 wild-type strain and unc-1(e538), ced-3(n1286), and ced-4(n1894) mutant strains used in this work were obtained from the Caenorhabditis Genetics Center (CGC, St. Paul, MN), which is funded by the National Institutes of Health, National Center for Research Resources (NCRR, Bethesda, MD).

General standard methods were used for the strain maintenance and culturing of C. elegans (22). In particular, animals were grown at 20 °C on NEM agar plates seeded with live bacteria (Escherichia coli strain OP50) as food source.

Statistics—Statistical analysis was done by unpaired Student’s t test with the exception of a comparison analysis of apoptotic cells in sel-12 mutants, which was done by one-way factorial analysis of variance.
Sensitivity of sel-12 Mutants to Paraquat—Age-synchronous hermaphrodites after maturation were prepared by the alkaline hypochlorite method (23). 2-day-old synchronized animals were placed on NG agar plates with OP50 and 40 μM 5-fluoroodeoxyuridine, which was used to block progeny development (24). Seven days after hatching, normally developed animals were transferred to NG agar plates containing 5 mM paraquat in addition to 5-fluoroodeoxyuridine and OP50. Animals were grown at 20 °C and subsequently followed for survival. On the fifth day after exposure to paraquat, the number of surviving animals was counted. The survival rate of 60 animals of each strain was followed for each of the four independent trials.

Sensitivity of sel-12 Mutants to Hydrogen Peroxide—Age-synchronous animals were grown in liquid culture (S medium, 0.001% cholesterol, and Escherichia coli strain NA22). Two days after hatching, hydrogen peroxide was added at several concentrations. After a 2-h exposure, the numbers of animals surviving was counted. The survival rate of 100 animals of each strain was followed for each of the four independent trials.

Cell Death Assays—Embryonic development was followed microscopically by using Nomarski optics, and apoptotic cells in the comma embryonic stage were counted. Embryos were collected by the alkaline hypochlorite method. The number of cell corpses was counted in 70 embryos for each of the three independent trials.

Dantrolene Treatment—Synchronized animals were grown on NG agar plates with 10 mM dantrolene. On the third day after hatching, embryos were collected by the alkaline hypochlorite method. Cell death in embryos was examined as described above.

RESULTS

sel-12(ar171) Mutants Show Resistance to Oxidative Stress—To investigate the relationship between oxidative stress and sel-12, we examined the survival rate of sel-12 mutants under oxidative stress. The toxicity of paraquat and hydrogen peroxide is exerted by their capacity to generate superoxide anions and hydroxy radicals (25, 26), which expose animals to oxidative stress. We treated animals with 5-fluoroodexyuridine to prevent the nematodes from dying from bags of worms (the sel-12 mutants have Egl phenotype and die from hatches of eggs in their bodies without using 5-fluoroodexyuridine). The survival rate is given in Fig. 1, A and B. Without paraquat, sel-12(ar171)unc-1(e538) showed a similar survival curve when compared with the control strain unc-1(e538) (Fig. 1A). In contrast, sel-12(ar171)unc-1(e538) showed an increased survival rate in the presence of 5 mM paraquat, and its survival rate at the fifth day after exposure to paraquat was significantly elevated compared with that of unc-1(e538) (Fig. 1, B and C), whereas the survival rate of sel-12(ar131) showed no significant change from that of unc-1(e538) (Fig. 1D). Treatment with 10 mM paraquat also caused sel-12(ar171) to show a tendency for higher resistance, although the difference was not statistically significant (data not shown). Similarly, sel-12(ar171)unc-1(e538) also showed elevated resistance to hydrogen peroxide compared with that of unc-1(e538) (Fig. 1D).

sel-12(ar171) Prevents mev-1(kn1)-induced Apoptosis—We constructed sel-12mev-1(kn1) double mutants to clarify the role of sel-12 in inducing apoptosis under endogenous oxidative stress and mitochondrial dysfunction. The number of apoptosis in embryos of C. elegans is easily identified by Nomarski optics (27, 28). The nucleus of the apoptotic cell becomes refractile resembling a flat button (Fig. 2A). The increased numbers of apoptosis were observed in mev-1(kn1) embryos (Fig. 2B). The number of apoptosis in sel-12(ar171)mev-1(kn1) embryos...
bryos was remarkably decreased, which means that sel-12(ar171) prevents mev-1-induced apoptosis (Fig. 2B).

The Number of Apoptosis in sel-12(ar131) Embryos Is Significantly Elevated—The number of apoptosis in sel-12(ar131)-unc-1(e538) was significantly increased as compared with that in unc-1(e538) (Fig. 3, A and B). The number of apoptosis in unc-1(e538), examined as a control in this study, was approximately the same as that in wild-type N2 (Fig. 3B). We next constructed ced-3(n1286)sel-12(ar131) and ced-4(n1894)sel-12(ar131) mutants to clarify the mechanism of sel-12(ar131)-induced cell death. The ced-3 product is homologous to the mammalian interleukin-1β-converting enzyme, which is essential for the execution of programmed cell death. The ced-4 product is a homologue of Apaf-1 and activates ced-3. Either of the ced-3 and ced-4 mutations eliminates programmed cell death (27, 29, 30). The examination of ced-3(n1286)sel-12(ar131) and ced-4(n1894)sel-12(ar131) embryos demonstrated that ced-3(n1286) and ced-4(n1894) inhibited sel-12(ar131)-induced apoptosis completely (Fig. 3, B and C), which means that sel-12(ar131)-induced apoptosis is dependent on ced-3 and ced-4 activity.

Dantrolene Treatment Prevents sel-12(ar131)-induced Apoptosis—Previous studies have indicated that perturbed Ca\(^{2+}\) release from the ER is a critical factor for FAD-related PS1-induced apoptosis (13, 31–33). Thus, we treated sel-12(ar131)-unc-1(e538) with dantrolene, which specifically inhibits Ca\(^{2+}\)
release from the ER stores. In embryos of dantrolene-treated sel-12(ar131)unc-1(e538), apoptosis was significantly decreased (Fig. 4), which suggests that, by abnormal regulation of calcium release from the ER, sel-12(ar131) predisposes embryo cells to apoptosis.

**DISCUSSION**

This study demonstrated the role of sel-12, a PS1 homologue of C. elegans, in apoptosis depending on oxidative stress from mitochondrial dysfunction. The sel-12(ar171) mutant, which genetically represents a null allele, confers increased resistance to paraquat and hydrogen peroxide. To investigate the role of sel-12 in apoptotic activities under oxidative stress from mitochondrial dysfunction, apoptosis in sel-12mev-1 mutant embryos was examined. The mev-1 gene encodes succinate dehydrogenase cytochrome b large subunit (Cyt-1), which is a component of complex II of the mitochondrial electron transport chain (34). This mutation elevated the
The Role of Presenilin-1 Homologue Gene sel-12

Effects of dantrolene against sel-12(ar131)-induced apoptosis. Age-synchronous matured hermaphrodites were treated with dantrolene to prevent Ca\(^{2+}\) release from the ER and reduce intracellular Ca\(^{2+}\). The embryos were then collected and scored for cell corpses. The number of apoptosis in sel-12(ar131)unc-10(e538) embryos was significantly reduced by dantrolene treatment. *p < 0.01 versus without dantrolene.

generation of reactive oxygen species from mitochondria (21) and increased the number of apoptosis as compared with wild-type N2 embryos. The sel-12(ar171)mev-1(0kn1) mutant showed a decreased number of apoptosis as compared with mev-1(0kn1), which means that sel-12(ar171) prevents mev-1-induced apoptosis. This is consistent with our findings that sel-12(ar171) is resistant to such oxidative stress agents as exogenoue paraquat or hydrogen peroxide.

On the other hand, in another allele mutant, sel-12(ar131) significantly increased the number of apoptosis as compared with that in wild type. Because the supernumerary apoptotic cells were suppressed in ced-3(n1286)sel-12(ar131) and ced-4(n1894)sel-12(ar131) embryos, this means that the sel-12(ar131)-induced apoptosis was ced-3- and ced-4-dependent.

sel-12(ar131) happens to have a conserved point mutation of PS1 C92S, which was reported by an Italian FAD family. Hence, it is expected that sel-12(ar131) has properties in common with the PS1 C92S mutation and with another FAD-linked mutant PS1.

Some studies have demonstrated that FAD-related mutant PS may sensitize neurons to apoptosis by perturbing intracellular calcium homeostasis (13). Accordingly, it is quite likely that sel-12(ar131) has a gain of proapoptotic function similar to that of the FAD-related mutant PS.

FIG. 4. Effects of dantrolene against sel-12(ar131)-induced apoptosis. The number of apoptosis in sel-12(ar131)unc-10(e538) embryos was significantly reduced by dantrolene treatment. *p < 0.01 versus without dantrolene.

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