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# Copper ions influence the toxicity of β-amyloid(1-42) in a concentration-dependent manner in a *Caenorhabditis elegans* model of Alzheimer's disease

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β-amyloid (Aβ) and copper play important roles in the pathogenesis of Alzheimer's disease (AD). However, the behavioral correlativity and molecular mechanisms of Aβ and copper toxicity have been investigated less often. In the present study, we investigated the interaction and toxicity of Aβ1-42 and copper in the Aβ1-42 transgenic *Caenorhabditis elegans* worm model CL2006. Our data show that the paralysis behavior of CL2006 worms significantly deteriorated after exposure to $10^{-3}$ mol $L^{-1}$ copper ions. However, the paralysis behavior was dramatically attenuated with exposure to $10^{-4}$ mol $L^{-1}$ copper ions. The exogenous copper treatment also partially changed the homeostatic balance of zinc, manganese, and iron. Our data suggest that the accumulation of reactive oxygen species (ROS) was responsible for the paralysis induced by Aβ and copper in CL2006. The ROS generation induced by Aβ and copper appear to be through *sod-1*, *prdx-2*, *skn-1*, *hsp-60* and *hsp-16.2* genes.

**Alzheimer's disease, β-amyloid(1-42), copper, *C. elegans*, oxidative stress**

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Senile plaques are a main hallmark of Alzheimer's disease (AD). As the main component of the senile plaque, Aβ1-42, which is short for β-amyloid peptide(1-42), is closely associated with the pathogenesis of AD [1,2]. Recent reports suggested that copper was involved in the pathogenesis of AD, although the underlying mechanism is very elusive [3]. Some *in vitro* studies suggested that the abnormal interactions of Aβ with copper are implicated in the formation of Aβ1-42 oligomers, which are more toxic than the monomers [4–6]. On the other hand, there is increasing evidence that oxidative stress is very strongly correlated with the pathogenesis of AD, although the exact mechanism is not clear [7]. There were some previous studies related to the role of copper in Aβ-generated transgenic rodent models, but otherwise the behavioral correlativity and molecular mechanism of Aβ and copper toxicity have been investigated less [8–10]. The current work explored the *in vivo* toxicity mechanism of Aβ1-42 and copper in the Aβ1-42 transgenic *Caenorhabditis elegans* model [11,12].

## 1 Materials and methods

### 1.1 Reagents

$CuCl_2$ (analysis grade) was dissolved in distilled/deionized (dd)-water and stored at 4°C. FUDR (5-fluoro-2′-deoxyuri-

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dine) and $H_2$DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate) were purchased from Sigma (Sigma-Aldrich, MO). Protein concentration was determined using the BCA kit (Thermo Scientific, Rockford, IL).

### 1.2 Worm strains and maintenance

Standard nematode growth medium (NGM) was used for the growth and maintenance of *C. elegans* at 20°C and 40% relative humidity condition [13,14]. Unless stated otherwise, plates were seeded with live *Escherichia coli* OP50 bacteria. Bristol N2 (Caenorhabditis Genetics Center, CGC) was used as the wild-type strain. The transgenic strain CL2006 (dvIs2[unc-54::human β-amyloid 1-42; pRF4]) containing the human Aβ(1-42) mimic-gene [12] was generated by Dr. Link of Colorado University (Boulder, CO, USA).

### 1.3 Paralysis assay

Synchronized hermaphroditic worm populations were transferred to plates with FUDR (100 mg $L^{-1}$), or various concentrations of $CuCl_2$ when the young adults began to lay eggs. On the first day of adulthood, 120 worms were placed on three plates for each treatment group. $CuCl_2$ stock solution (1 mol $L^{-1}$) was diluted into a live *E. coli* OP50 suspension, reaching the indicated final concentrations at $10^{-3}$, $10^{-4}$, or $10^{-6}$ mol $L^{-1}$, and was placed on the surface of the NGM plates. The worms were tested for paralysis by tapping their noses with a platinum wire. Worms that moved their noses but failed to move their bodies were scored as "paralyzed". To avoid scoring of old worms as paralyzed, the paralysis assay was terminated on day 12 of adulthood [15].

### 1.4 Biodistribution assay of elements with micro-beam synchrotron radiation X-ray fluorescence (μ-SRXRF)

Worms were maintained and treated as described above. The worms were then transferred to fresh treatment plates every two days for the first 12 d. Worms were harvested on different days of adulthood and they were washed five times with dd-water. The washed worms were placed on polycarbonate film after being fixed on a plastic frame and quickly frozen in liquid nitrogen for a few seconds. Then the worms were kept at room temperature until the subsequent μ-SRXRF measurement at the Beijing Synchrotron Radiation Facility. The distributions of elements Cu, Zn, Mn, and Fe were determined. The spectra were analyzed by the AXIL program. The concentration was calculated by means of the normalization of the Compton scattering intensity [16].

### 1.5 Measurement of the level of ROS in *C. elegans*

The level of ROS (reactive oxygen species) in *C. elegans* was measured by the $H_2$DCF-DA assay [17]. Worms were maintained and treated as described above. The worms were then transferred to fresh treatment plates every two days for the first 12 d of the assay. Worms were harvested on different days of adulthood and were washed off the plates with cold M9 buffer. After three washes, the worms were re-suspended in M9 buffer. Hundred μL volume of the suspension was aliquoted into four replicate wells of a 96-well plate, and allowed to equilibrate to room temperature. In the meantime, a fresh 2 μL H2-DCF-DA solution was pipetted into the suspensions to reach a final 100 μmol $L^{-1}$ concentration. Worms without H2-DCF-DA, and H2-DCF-DA without worms were used as negative controls. After addition of H2-DCF-DA, basal fluorescence was measured in a Thermo Labsystems Fluoroskan Ascent Microplate Reader at excitation/emission wavelengths of 485 and 520 nm. The initial fluorescence signals of the control wells were subtracted from the corresponding signals of each well after the second measurement. 1 mL of the initial worm suspension from each sample was kept at −80°C for later protein quantification to normalize the fluorescence signal. Assays were performed in independent duplicate experiments.

### 1.6 Gene expression analysis with quantitative real-time PCR

Worms were maintained and treated as described above. The worms were then transferred to fresh treatment plates every two days for the first 12 d. Worms were harvested on different days of adulthood and were washed five times with deionized water. Total RNA was extracted from adult worms with TRIzol reagent (Invitrogen), and cDNA was produced by SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen) [18]. The primers are listed in Table 1. The ama-1 was used as the internal control. Quantitative RT-PCR was carried out on a Rotor-Gene 6000 centrifugal real-time cycler (Corbett Research, CA) using the Platinum SYBR Green qPCR SuperMix-UDG with ROX. The cycling conditions were as follows: 50°C for 2 min, initial denaturation at 95°C for 2 min, followed by 45 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C.

### 1.7 Quantification and data analysis

Statistical analyses were performed by one-way ANOVA and shown as mean±SE. Experiments were performed in triplicate except when stated otherwise. Differences were considered statistically significant at $P<0.05$.

## 2 Results

### 2.1 High concentration copper increased paralysis, and low concentration copper decreased paralysis in CL2006

Previous reports have suggested that copper is involved in

**Table 1** The quantitative real-time PCR primer information

| Genes | Primers | Sequence (5'→3') |
|---|---|---|
| *ama-1* | Forward | CTGACCCAAAGAACACGGTGA |
| | Reverse | TCCAATTCGATCCGAAGAAGC |
| *sod-1* | Forward | ACGCTTTACGGTCCAAACACT |
| | Reverse | CTTGGACTCTTCTGCCTTGTCT |
| *sod-2* | Forward | AAACAGCTTTCGGCATCAAC |
| | Reverse | TTCCGAACAGTGGAACAAGTC |
| *sod-3* | Forward | AGAACCTTCAAAGGAGCTGATG |
| | Reverse | CCGCAATAGTGATGTCAGAAAG |
| *ctl-1* | Forward | GCGGATACCGTACTCGTGAT |
| | Reverse | GTGGCTGCTCGTAGTTGTGA |
| *ctl-2* | Forward | TCCGTGACCCTATCCACTTC |
| | Reverse | TGGGATCCGTATCCATTCAT |
| *ctl-3* | Forward | GCGGATACCGTACTCGTGAT |
| | Reverse | GTGGCTGCTCGTAGTTGTGA |
| *prdx-2* | Forward | TCTTCATCATCGACCCATCA |
| | Reverse | CAAACCTCTCCGTGCTTCTC |
| *C11E4.1* | Forward | ATACCGTGGACAGGTGCTTC |
| | Reverse | CATGGGAAGGCAATGAGAGT |
| *skn-1* | Forward | AGTGTCGGCGTTCCAGATTTC |
| | Reverse | GTCGACGAATCTTGCGAATCA |
| *hsf-1* | Forward | ATGACTCCACTGTCCCAAGG |
| | Reverse | TCTTGCCGATTGCTTTCTCT |
| *hsp-60* | Forward | TTCAAGTCGTCGCAATCAAG |
| | Reverse | TCGACTTCTCCGAGATCGTT |
| *hsp-16.2* | Forward | CTCAACGTTCCGTTTTTGGT |
| | Reverse | CGTTGAGATTGATGGCAAAC |

the pathogenesis of AD [3], but most of the studies used high concentrations of copper, and studies using low concentrations of copper on animal models were not found. Therefore, we studied the effect of different concentrations of copper on the behavior of the CL2006 worm. The behavioral assay indicated that treatment with a high concentration ($10^{-3}$ mol $L^{-1}$) of copper significantly accelerated the paralysis rate of the CL2006 worm, and a low concentration ($10^{-4}$ mol $L^{-1}$) copper dramatically decelerated the paralysis rate of the CL2006 worm on the 8th day of adulthood. The lower concentration ($10^{-6}$ mol $L^{-1}$) copper treatment had no significant effect on the paralysis rate of the CL2006 worm. At the same time, we also studied the effect of different concentrations of copper on the behavior of the wild type worm N2 (no Aβ) and we found that there was no paralysis behavior in any of them. These results show that copper influenced the Aβ1-42 toxicity in a biphasic concentration-dependent manner (Figure 1).

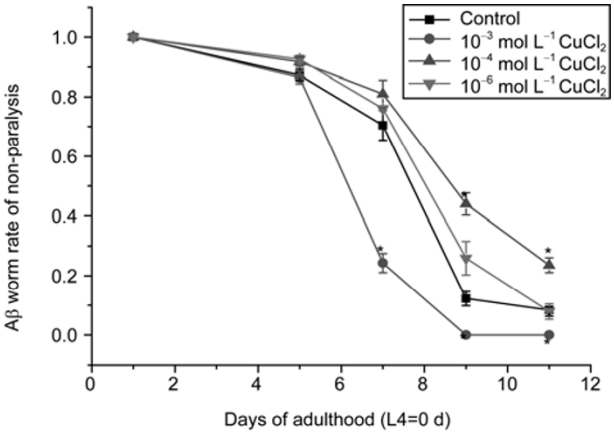


**Figure 1** Dose response of copper on Aβ toxicity in the transgenic worm (CL2006, the Aβ worm) using a paralysis test. The synchronized hermaphroditic worm populations were transferred to plates with FUDR (100 mg $L^{-1}$) with various concentrations of $CuCl_2$ when the young adults began to lay eggs. The worms were tested for paralysis by tapping their noses with a platinum wire. Worms that moved their noses but failed to move their bodies were scored as "paralyzed". Statistical analyses were performed and shown as mean±SE, $n$=120; *, $P$<0.05 significantly different from the control group. The control group was CL2006. $10^{-3}$ mol $L^{-1}$ $CuCl_2$ group indicates that CL2006 was treated with $CuCl_2$ at 1 mmol $L^{-1}$ concentration. There were no dose-dependent responses of copper on the paralysis behavior in the wild-type worm N2 (the rate of paralysis is 0, data not shown).

## 2.2 Aβ1-42 facilitated copper absorption in a concentration-dependent manner and mediated other metal homeostasis in the worms

To study the behavioral correlativity of copper toxicity, the copper concentration and homeostasis of other related metals in different parts of the worm body were detected by micro-beam synchrotron radiation X-ray fluorescence (μ-SRXRF). It was found that the copper mass percent of the CL2006 was significantly elevated in the head part and the middle part of the worm on about the 4th day of adulthood after high concentration ($10^{-3}$ mol $L^{-1}$) copper treatment. However, the copper mass percent of the CL2006 worm did not change significantly in these parts mentioned above (Figure 2) after the low concentration ($10^{-4}$ and $10^{-6}$ mol $L^{-1}$) copper treatments.

A previous report has shown that zinc, manganese, and iron are associated with the pathogenesis of AD [19]. Therefore, the effects of copper administration on the homeostasis of these metals were measured. The zinc mass percent of the CL2006 worm was significantly elevated just in the head part on about the 4th day of adulthood (result not shown) after the low concentration ($10^{-4}$ and $10^{-6}$ mol $L^{-1}$) copper treatments, while the manganese mass percent of the CL2006 worm was significantly elevated just in the head part on about the 4th day of adulthood (result not shown). The iron mass percent of the CL2006 worm was significantly elevated just in the head part on about the 4th day of adulthood after the high concentration ($10^{-3}$ mol $L^{-1}$) copper treatment (result not shown).

These results indicate that after copper treatment, the copper mass percent of the CL2006 worm is significantly elevated in the head part and the middle part, mainly on about the 8th day of adulthood. With regard to the other three important oxidative stress-related metals, zinc, manganese, and iron, their changes were very local (just in head)

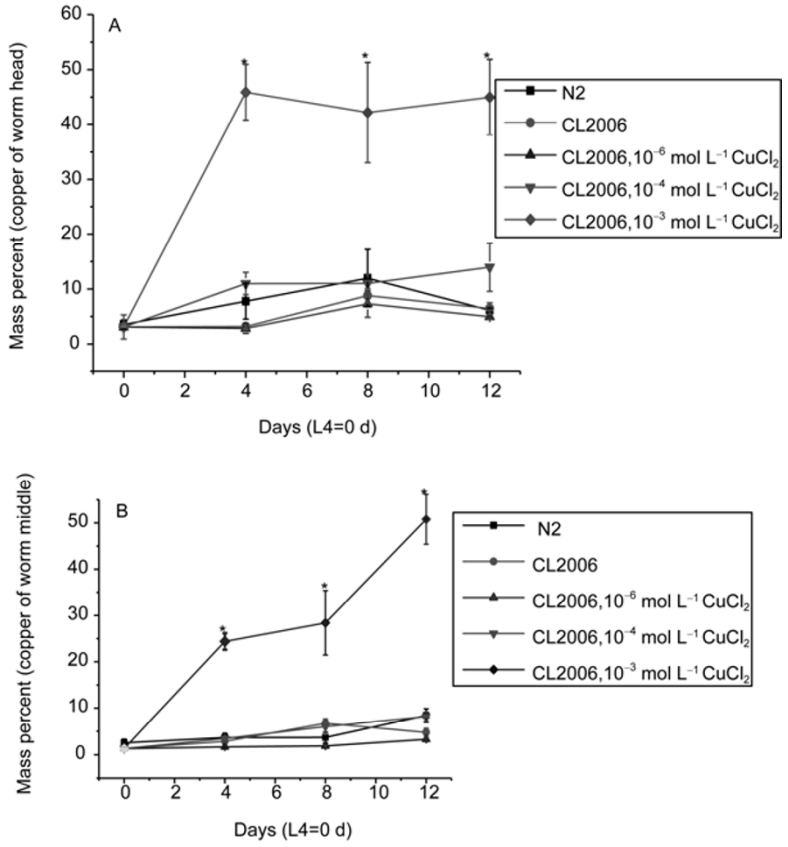


**Figure 2** Copper concentration and other metal homeostasis in different parts of the worms were detected by micro-beam synchrotron radiation X-ray fluorescence (μ-SRXRF). Statistical analyses were performed and shown as mean±SE, $n$=120; *, $P$<0.05 significantly different from the control group. The control group was CL2006.

and not significantly associated with the worm paralysis behavior, although copper treatment affected their homeostasis in the CL2006 worm.

### 2.3 High concentration copper increased the level of ROS, and low concentration copper decreased the level of ROS in CL2006

Some studies reported that oxidative stress is involved in the pathogenesis of the AD. Metal toxicity and the correlativity of metal ions and oxidative stress in AD behavioral phenotypes were not clear [8−10]. Based on the behavioral assay and metal element analysis described above, the level of ROS in the worms was measured by $H_2$DCF-DA assay to evaluate the behavioral correlativity and molecular mechanism of copper toxicity. It was found that the level of ROS in the CL2006 worm was significantly higher than the wild type worm N2 (no Aβ) on the 4th, 8th, and 12th days of adulthood. This indicated that Aβ1-42 was involved in the generation of ROS in the CL2006 worm. Thus, it was found that the level of ROS in the CL2006 worm was significantly elevated by 75% on about the 4th day of adulthood after treatment with the high concentration ($10^{-3}$ mol $L^{-1}$) copper. However, the level of ROS in the CL2006 worm was significantly lowered by 50% on about the 8th day of adulthood after low concentration ($10^{-4}$ mol $L^{-1}$) copper treatment. The level of ROS in the CL2006 worm was not significantly changed after lower concentration ($10^{-6}$ mol $L^{-1}$) copper treatment (Figure 3). On about the 8th day of adulthood, $10^{-4}$ mol $L^{-1}$ copper treatment significantly decreased the level of ROS in the CL2006 worm even more than the $10^{-6}$ mol $L^{-1}$ copper treatment, compared with the control group (CL2006 worm), but the $10^{-3}$ mol $L^{-1}$ copper treatment significantly increased the level of ROS in the CL2006 worm. These results suggest that oxidative stress was involved in the behavioral changes induced by copper in a concentration-dependent manner in the CL2006 worm. Thus, they suggest an interaction between copper and Aβ1-42 could exist, and could be involved in the behavioral changes induced by copper in a concentration-dependent manner in the CL2006 worm.

### 2.4 Copper incorporation significantly changed the RNA levels of *sod-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, and *skn-1* genes in CL2006

Because oxidative stress appeared to be related to the behavioral changes caused by copper in the CL2006 worm, we

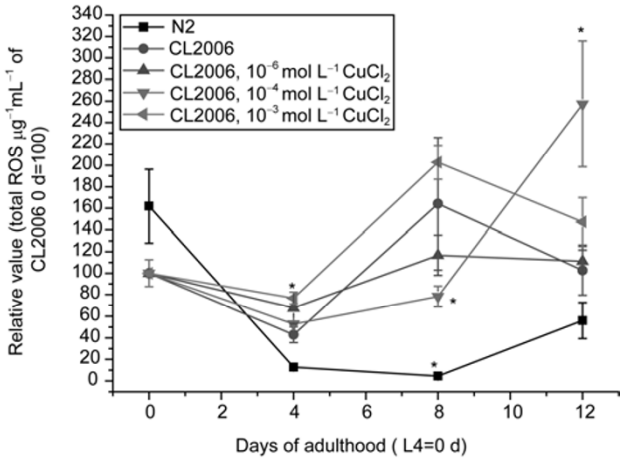


**Figure 3** Intracellular levels of ROS in worms. The synchronized hermaphroditic worm populations were transferred to plates with FUDR (100 mg $L^{-1}$) or various concentrations of $CuCl_2$ when the young adults began to lay eggs. The level of ROS in *C. elegans* was measured by $H_2$DCF-DA assay. Statistical analyses were performed and shown as mean±SE, $n$=120; *, $P$<0.05 significantly different from the control group. The control group was CL2006.

analyzed the expression levels of several oxidative stress genes [20–28] at the 8th day of adulthood. We compared gene expression levels between the wild type worm N2 and the Aβ1-42-transgenic worm CL2006. Results demonstrated that the expression level of the genes *sod-2*, *ctl-1*, *ctl-3*, *hsf-1*, *C11E4.1* and *skn-1* in N2 was significantly higher than the expression level of these genes in CL2006. We also compared gene expression level in the CL2006 worm with $10^{-6}$, $10^{-4}$ and $10^{-3}$ mol $L^{-1}$ $CuCl_2$ treatment, respectively, compared with the CL2006 worm without $CuCl_2$ treatment. Results showed that the expression level of *sod-1*, *sod-2*, *ctl-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, *C11E4.1*, and *skn-1* genes in the CL2006 worm with $CuCl_2$ treatment ($10^{-6}$, $10^{-4}$ or $10^{-3}$ mol $L^{-1}$) was significantly different from that of the CL2006 worm without $CuCl_2$ treatment, which suggested that *sod-1*, *sod-2*, *ctl-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, *C11E4.1*, and *skn-1* genes probably are involved in the interaction and toxicity between Aβ1-42 and the different copper concentrations in the CL2006 worm. More precisely, heat shock factor hsf-1, mitochondria-stress response factor hsp-60, and the endoplasmic reticulum-stress response factor hsp-16.2 were significantly increased after high concentration ($10^{-3}$ mol $L^{-1}$) copper treatment in the CL2006 worm. However, superoxide dismutase *sod-1,2,3*, catalase *ctl-1,2,3*, thioredoxin reductase *prdx-2*, glutathione peroxidase *C11E4.1*, and the oxidative stress regulator *skn-1* were increased after low concentration ($10^{-4}$ and $10^{-6}$ mol $L^{-1}$) copper treatment in the CL2006 worm (Figure 4). It was noteworthy that the RNA levels of *sod-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, and *skn-1* genes were significantly changed in the CL2006 worm with different concentrations of copper treatment.

## 3 Discussion

Markesbery *et al.* [7] previously raised the oxidative stress hypothesis in AD. Most reports indicated that oxidative stress was very closely related to the pathogenesis of AD [29,30]. However, these results could not confirm that oxidative stress was either a cause or a consequence of the pathogenesis of AD [31–33]. The behavioral assay and ROS detection in our study showed that the alteration of ROS level proceeded before the exacerbation of paralysis after copper treatment. This result suggests that elevated ROS level is an important factor in the paralysis behavior of the CL2006 worm which is an Aβ1-42 transgenic *C. elegans* model of AD. Thus, this result indicates that oxidative stress is an important factor in the pathogenesis of AD [19].

Bush *et al.* [34,35] reported that Aβ1-42 toxicity in AD may be caused by abnormal interactions with metal ions such as copper, and provided a "metallobiology" model for the pathogenesis of AD. Nevertheless, there were some studies to support copper overload as the underlying mechanism in AD, while there were some studies that indicated copper deficiency as the relevant mechanism in AD [36]. Both the behavioral assay and element analysis results in our study showed that high concentrations of copper can significantly increase the paralysis rate of the CL2006 worm and low concentrations of copper can significantly decrease paralysis rate of the CL2006 worm. This suggests that high levels of copper or low levels of copper both can affect the Aβ1-42 toxicity which is involved in the pathogenesis of AD.

To elucidate how copper ions are involved in the regulation of ROS levels and gene expression levels in the Aβ1-42 transgenic *C. elegans* model of AD, we synthesized the results in the present study and made comprehensive conclusions. On about the 8th day of adulthood, the ROS level in N2 was significantly lower than the ROS level in CL2006, while the expression levels of the genes *sod-2*, *ctl-1*, *ctl-3*, *hsf-1*, *C11E4.1* and *skn-1* in N2 were significantly higher than those of these genes in CL2006. Thus, *sod-2*, *ctl-1*, *ctl-3*, *hsf-1*, *C11E4.1* and *skn-1* genes may be involved in the Aβ1-42-induced paralysis behavior of the CL2006 worm (Figure 4). Moreover, we compared the similarity and consistency between the paralysis rate test and ROS level test of the CL2006 worm, as well as the expression pattern of some genes on about the 8th day of adulthood when the CL2006 worm was treated with $10^{-6}$, $10^{-4}$ , $10^{-3}$ mol $L^{-1}$ $CuCl_2$ and without $CuCl_2$ treatment, respectively (Table 2). We paid attention to the significant changes of expression patterns of some genes when the CL2006 worm was treated with $CuCl_2$ at concentrations between $10^{-4}$ and $10^{-3}$ mol $L^{-1}$. It was found that *sod-1*, *sod-2*, *ctl-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, *C11E4.1*, and *skn-1* genes probably are involved in the interaction and toxicity between Aβ1-42 and copper in the CL2006 worm. Specifically, *sod-1*, *prdx-2*, and *skn-1*

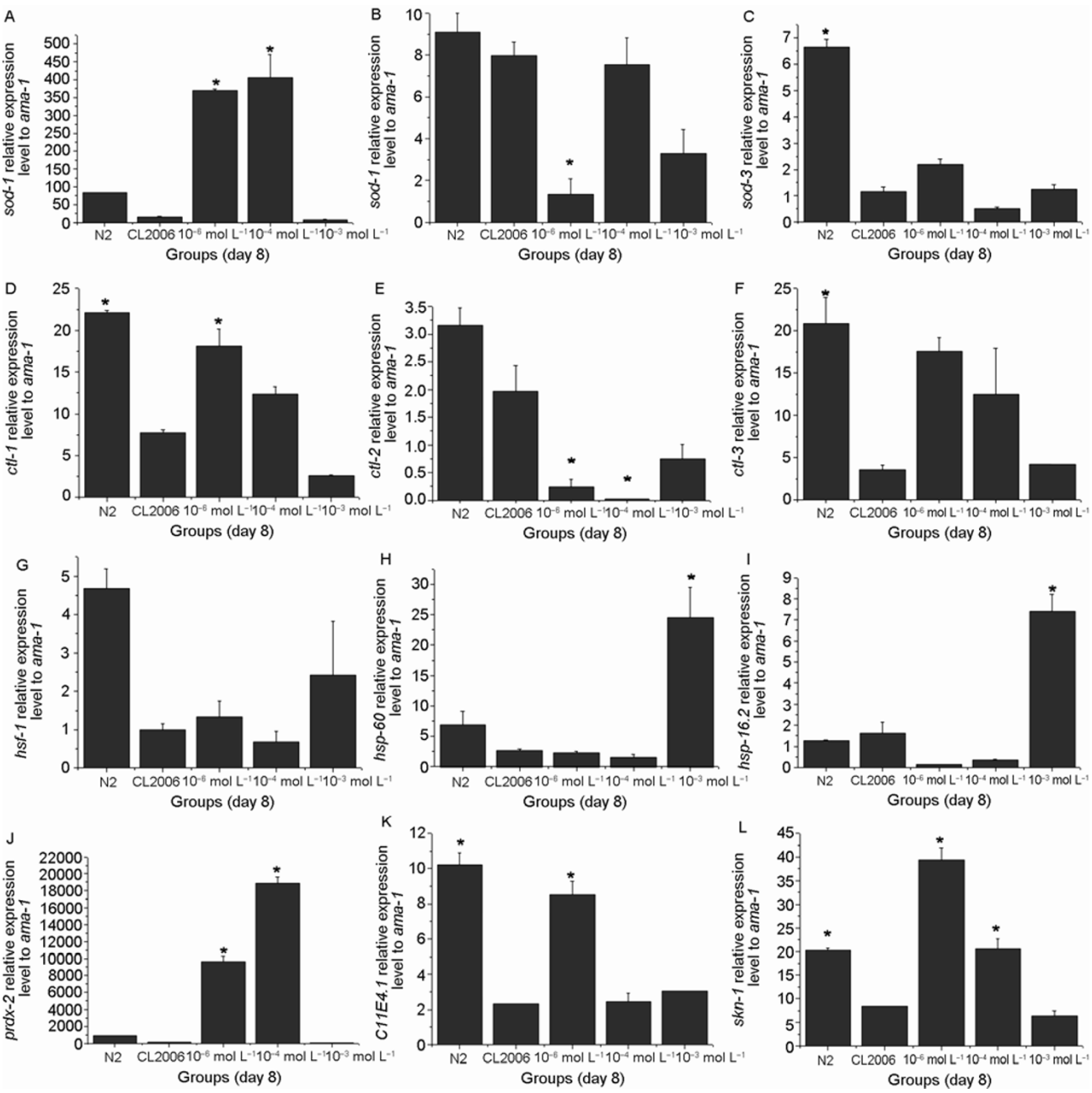


**Figure 4** Related gene expression level relative to *ama-1*. A, *sod-1*. B, *sod-2*. C, *sod-3*. D, *ctl-1*. E, *ctl-2*. F, *ctl-3*. G, *hsf-1*. H, *hsp-60*. I, *hsp-16.2*. J, *prdx-2*. K, *C11E4.1*. L, *skn-1*. The synchronized hermaphroditic worm populations were transferred to plates with FUDR (100 mg $L^{-1}$) or various concentrations of $CuCl_2$ when the young adults began to lay eggs. Statistical analyses were performed and shown as mean±SE, $n$=120; *, $P$<0.05 significantly different from the control group. The control group was CL2006, $10^{-3}$, $10^{-4}$, or $10^{-6}$ mol $L^{-1}$.

genes are perhaps responsible for the lower ROS level and paralysis rate of the CL2006 worm with the $10^{-4}$ mol $L^{-1}$ $CuCl_2$ treatment. However, both the *hsp-60* and *hsp-16.2* genes are perhaps responsible for the higher ROS level and paralysis rate of the CL2006 worm with $10^{-3}$ mol $L^{-1}$ $CuCl_2$ treatment. Together, we found that this group of genes, *sod-1*, *prdx-2*, and *skn-1*, as well as the other group of genes, *hsp-60* and *hsp-16.2*, were involved in the interaction and toxicity between Aβ1-42 and copper in a concentration-dependent manner in the CL2006 worm. In summary, the high expression levels of *sod-1*, *prdx-2*, and *skn-1* genes are associated with the scavenging of ROS and ameliorating the paralysis behavior of the CL2006 worm, while high expression levels of *hsp-60* and *hsp-16.2* genes are related to increased ROS level and the deteriorating paralysis behavior of the CL2006 worm.

In conclusion, in the present study, we investigated the *in vivo* toxicity of Aβ(1-42) and copper interaction in the Aβ(1-42) transgenic *C. elegans* worm model. Our data suggest that high concentrations of copper significantly in-

**Table 2** Outline of similarity and consistency between paralysis rate test and ROS level test of the CL2006 worm, as well as the expression pattern of some genes[a)]

| | CL2006 | $10^{-6}$ mol $L^{-1}$ $CuCl_2$ | $10^{-4}$ mol $L^{-1}$ $CuCl_2$ | $10^{-3}$ mol $L^{-1}$ $CuCl_2$ | Remarks |
|---|---|---|---|---|---|
| Paralysis rate | 0 | –1 | –2 | 1 | |
| ROS level | 0 | –1 | –2 | 1 | |
| *sod-1* | 0 | 1 | 2 | –1 | *, a |
| *sod-2* | 0 | –3 | –1 | –2 | *, a |
| *sod-3* | 0 | 1 | –2 | –1 | |
| *ctl-1* | 0 | 2 | 1 | –1 | *, b |
| *ctl-2* | 0 | –2 | –3 | –1 | *, c |
| *ctl-3* | 0 | 3 | 2 | 1 | |
| *hsf-1* | 0 | 1 | –1 | 2 | |
| *hsp-60* | 0 | –1 | –2 | 1 | *, d |
| *hsp-16.2* | 0 | –2 | –1 | 1 | *, d |
| *prdx-2* | 0 | 1 | 2 | –1 | *, a |
| *C11E4.1* | 0 | 3 | 1 | 2 | *, b |
| *skn-1* | 0 | 2 | 1 | –1 | *, b |

a) The paralysis rate, ROS level, and gene expression level of the CL2006 worm without copper treatment were regarded as the baseline, which are indicated by the number 0. The numbers 1, 2, and 3 indicate the degree when paralysis rate, ROS level, and gene expression level are more than the baseline, respectively. The numbers –1, –2, and –3 indicate the degree when paralysis rate, ROS level, and gene expression level are less than the baseline, respectively. The symbol * indicates significance of gene expression level compared with the baseline, including *sod-1*, *sod-2*, *ctl-1*, *ctl-2*, *hsp-60*, *hsp-16.2*, *prdx-2*, *C11E4.1*, and *skn-1* genes; a, b, c and d indicate changing pattern of gene expression level according to the order of $10^{-6}$, $10^{-4}$ and $10^{-3}$ mol $L^{-1}$ $CuCl_2$ treatment. There are *sod-1*, *sod-2*, and *prdx-2* in pattern a, *ctl-1*, *C11E4.1* and *skn-1* in pattern b, *ctl-2* in pattern c, *hsp-60* and *hsp-16.2* in pattern d.

crease the paralysis rate of the Aβ(1-42) worm, and low concentrations of copper significantly decrease the paralysis rate of the Aβ(1-42) worm. This behavioral correlativity caused by the Aβ(1-42) and copper interaction is mainly through reactive oxygen species (ROS), which appear to be regulated by the changing expression intensity of *sod-1*, *prdx-2*, *skn-1*, *hsp-60*, and *hsp-16.2* genes. These novel findings provide a molecular mechanism for the pathogenesis of AD.

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1 Mattson M P. Pathways towards and away from Alzheimer's disease. Nature, 2004, 430: 631–639
2 Goedert M, Spillantini M G. A century of Alzheimer's disease. Science, 2006, 314: 777–781
3 Bush A I. The metallobiology of Alzheimer's disease. Trends Neurosci, 2003, 26: 207–214
4 Bush A I, Curtain C C. Twenty years of metallo-neurobiology: Where to now? Eur Biophys J, 2007, 37: 241–245
5 Cater M A, McInnes K T, Li Q X, *et al*. Intracellular copper deficiency increases amyloid-β secretion by diverse mechanisms. Biochem J, 2008, 412: 141–152
6 Crouch P J, Hung L W, Adlard P A, *et al*. Increasing Cu bioavailability inhibits Aβ oligomers and tau phosphorylation. Proc Natl Acad Sci USA, 2009, 106: 381–386
7 Markesbery W R. Oxidative stress hypothesis in Alzheimer's disease. Free Radical Bio Med, 2007, 23: 134–147
8 Sparks D L, Schreurs B G. Trace amounts of copper in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. Proc Natl Acad Sci USA, 2003, 100: 11065–11069
9 Phinney A L, Drisaldi B, Schmidt S D, *et al*. *In vivo* reduction of amyloid-β by a mutant copper transporter. Proc Natl Acad Sci USA, 2003, 100: 14193–14198
10 Bayer T A, Schäfer S, Simons A, *et al*. Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Aβ production in APP23 transgenic mice. Proc Natl Acad Sci USA, 2003, 100: 14187–14192
11 Link C D. Invertebrate models of Alzheimer's disease. Genes Brain Behav, 2005, 4: 147–156
12 Link C D. *C. elegans* models of age-associated neurodegenerative diseases: Lessons from transgenic worm models of Alzheimer's disease. Exp Gerontol, 2006, 41: 1007–1013
13 Brenner S. The genetics of *Caenorhabditis elegans*. Genetics, 1974, 77: 71–94
14 Feng Z, Li W, Ward A, *et al*. A *C. elegans* model of nicotine-dependent behavior: Regulation by TRP-family channels. Cell, 2006, 127: 621–633
15 Cohen E, Bieschke J, Perciavalle R M, *et al*. Opposing activities protect against age-onset proteotoxicity. Science, 2006, 313: 1604–1610
16 Gao Y X, Liu N Q, Chen C Y, *et al*. Mapping technique for biodistribution of elements in a model organism, *Caenorhabditis elegans*, after exposure to copper nanoparticles with microbeam synchrotron radiation X-ray fluorescence. J Anal At Spectrom, 2008, 23: 1121–1124
17 Schulz T J, Zarse K, Voigt A, *et al*. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab, 2007, 6: 280–293
18 Hoogewijs D, Houthoofd K, Matthijssens F, *et al*. Selection and validation of a set of reliable reference genes for quantitative sod gene expression analysis in *C. elegans*. BMC Mol Biol, 2008, 9: 9
19 Praticò D. Oxidative stress hypothesis in Alzheimer's disease: A reappraisal. Trends Pharmacol Sci, 2008, 29: 609–615
20 Giglio A M, Hunter T, Bannister J V, *et al*. The copper/zinc superoxide dismutase gene of *Caenorhabditis elegans*. Biochem Mol Biol Int, 1994, 33: 41–44
21 Hunter T, Bannister W H, Hunter G J. Cloning, expression, and characterization of two manganese superoxide dismutases from *Caenorhabditis elegans*. J Biol Chem, 1997, 272: 28652–28659
22 Taub J, Lau J F, Ma C, *et al*. A cytosolic catalase is needed to extend adult lifespan in *C. elegans* daf-C and clk-1 mutants. Nature, 1999,

399: 162–166
23 Isermann K, Liebau E, Roeder T, *et al*. A peroxiredoxin specifically expressed in two types of pharyngeal neurons is required for normal growth and egg production in *Caenorhabditis elegans*. J Mol Biol, 2004, 338: 745–755
24 Simonetta S H, Romanowski A, Minniti A N, *et al*. Circadian stress tolerance in adult *Caenorhabditis elegans*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 2008, 194: 821–828
25 An J H, Vranas K, Lucke M, *et al*. Regulation of the *Caenorhabditis elegans* oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. Proc Natl Acad Sci USA, 2005, 102: 16275–16280
26 Steinkraus K A, Smith E D, Davis C, *et al*. Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *Caenorhabditis elegans*. Aging Cell, 2008, 7: 394–404
27 Benedetti C, Haynes C M, Yang Y, *et al*. Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. Genetics, 2006, 174: 229–239
28 Hong M, Kwon J Y, Shim J, *et al*. Differential hypoxia response of hsp-16 genes in the nematode. J Mol Biol, 2004, 344: 369–381
29 Good P F, Werner P, Hsu A, *et al*. Evidence for neuronal oxidative damage in Alzheimer's disease. Am J Pathol, 1996, 149: 21
30 Markesbery W R, Carney J M. Oxidative alterations in Alzheimer's disease. Brain Pathology, 1999, 9: 133–146
31 Smith M A, Rottkamp C A, Nunomura A, *et al*. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta—Mol Basis Dis, 2000, 1502: 139–144
32 Zhu X, Raina A K, Perry G, *et al*. Alzheimer's disease: The two-hit hypothesis. Lancet Neurol, 2004, 3: 219–226
33 Perry G, Nunomura A, Hirai K, *et al*. Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radical Bio Med, 2002, 11: 1475–1479
34 Lovell M A, Robertson J D, Teesdale W J, *et al*. Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci, 1998, 158: 47–52
35 Huang X, Cuajungco M P, Atwood C S, *et al*. Cu(II) potentiation of Alzheimer Aβ neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem, 1999, 274: 37111–37116
36 Quinn J F, Crane S, Harris C, *et al*. Copper in Alzheimer's disease: Too much or too little? Expert Rev Neurother, 2009, 9: 631–637