***Caenorhabditis elegans* nuclear hormone receptor NHR-14, cooperates with p53/cep-1 to regulate DNA damage-induced apoptosis**

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Keywords: *nhr-14*; DNA damage; apoptosis

Received: September 28, 2017

Accepted: December 05, 2017

Published: January 03, 2018

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ABSTRACT

Nuclear hormone receptor is involved in transcription regulation and many important cellular processes including development and metabolism. However, the role of nuclear hormone receptor in DNA damage-induced apoptosis remains elusive. Here we reported that RNAi of *nhr-14*, which was thought to be an estrogenic hormone receptor in *Caenorhabditis elegans*, inhibited DNA damage-induced apoptosis in *prmt-5(gk357)*, a *C. elegans* homolog of mammalian type II arginine methyltransferase PRMT5, after ionizing radiation. Deletion of *nhr-14* led to decreased DNA damage-induced germline apoptosis, but not in the physiological programmed cell death. We also demonstrate that *nhr-14* is not a checkpoint gene and functions downstream of the checkpoint pathway. Moreover, we show that *nhr-14* regulates *egl-1* and *ced-13* transcription upon DNA damage. In addition, we provided evidence that NHR-14 forms a complex with CEP-1/p53 and may function as a cofactor of CEP-1/p53. These findings indicate that NHR-14 might cooperate with CEP-1/p53 to regulate DNA damage-induced apoptosis, which reveals a novel role for nuclear hormone receptor in apoptosis.

INTRODUCTION

Programmed cell death (i.e., apoptosis) is one of the most important processes in the metazoans development. It plays the key roles in animal development and DNA damage repair. DNA damage-induced apoptosis is the cell death happening after severe DNA damage, which is associated with a number of human diseases including cancer. *Caenorhabditis elegans* has been used extensively to study the programmed cell death induced by DNA

damage responses. In *C. elegans*, the p53 homolog CEP-1 acts as a key effector to mediate germ cell apoptosis triggered by ionizing irradiation [1]. Although many factors have been reported to be involved in p53/cep-1 dependent apoptotic pathway, the details of this pathway is still not completely understood.

Nuclear hormone receptors (NHRs) comprise of a large family of transcription factors distinguished by a highly conserved DNA binding domain and a structurally conserved ligand-binding domain. There are 284 predicted

NHR genes in *C.elegans* [2]. Nuclear hormone receptors have been shown to regulate important developmental process [3–6]. However, the role of NHR in programmed cell death has not been documented.

We previously demonstrated that *prmt-5*, the *C. elegans* homolog of mammalian type II arginine methyltransferase PRMT5, negatively regulates DNA damage-induced apoptosis [7]. *prmt-5(gk357)* deletion mutants have increased germline programmed cell death after DNA damage. Furthermore, genetic analyses indicated that *prmt-5*-mediated apoptosis depends on *cep-1/p53* and requires the core cell death pathway.

In the present study, we show that RNAi knockdown of *nhr-14/HNF4*, which is thought to be an estrogen receptor in *C. elegans*[8], suppresses DNA damage-induced apoptosis in *prmt-5(gk357)* deletion mutant. Further, we show that *nhr-14/HNF4* is a new factor involved in the DNA damage-induced apoptosis and that *nhr-14* is not a checkpoint gene and functions downstream of the checkpoint genes. Our study confirmed that NHR-14/HNF4, cooperates with CEP-1/p53 to regulate *egl-1* (Bcl-2 homology region 3 domain containing gene) and *ced-13* (Bcl-2 homology region 3 domain containing gene) expression and DNA damage-induced apoptosis, which may offer clinical target for cancer therapy.

RESULTS

Inactivation of *nhr-14/HNF4* inhibits DNA damage-induced apoptosis

To examine whether nuclear hormone receptor is directly involved in the regulation of DNA damage-induced apoptosis, we performed RNAi screen in the background of *prmt-5(gk357)*. We found that knockdown of *nhr-14/HNF4* RNAi reduced the DNA damage-induced programmed cell death in *prmt-5(gk357)* (Figure 1A) after ionizing irradiation.

C. elegans nhr-14 gene is defined by the open reading frame T01B10.4 located on the linkage group X, which encodes a protein of 435 amino acids. *nhr-14(tm1473)* contains a deletion of 409bp in the third exon and intron resulting in an early stop of NHR-14 translation [8].

In order to test the function of *nhr-14/HNF4* in DNA damage-induced apoptosis, we used *nhr-14(tm1473)* deletion mutant to analyze the germ cell apoptosis after ionizing irradiation, we found that *nhr-14(tm1473)* can inhibit DNA damage-induced apoptosis in *prmt-5(gk357)* at different gamma-irradiation dose (Figure 1B) and different time (Figure 1C), suggesting that *nhr-14* functions downstream of *prmt-5* and regulates DNA damage-induced programmed cell death.

To further determine whether *nhr-14/HNF4* is a new factor that involved in the DNA damage induced cell apoptosis, we performed epistasis analysis using several well-defined cell survival molecules including

AKT-1/AKT, ABL-1/ABL and CED-9/BCL2. Previous studies have demonstrated that loss function mutation of *C.elegans akt-1(ok525)* exhibits dramatic increased programmed cell death after gamma-irradiation [9] and that mutation of *abl-1/abl1* induces more germline apoptosis than wildtype. Furthermore, it has been shown that loss function of *ced-9*, a BCL-2 homolog in *C.elegans* [10], activates CED-3 to induce programmed cell death [11, 12] and that *ced-9(n1653)* mutant exhibits more apoptotic cells upon DNA damage treatment. Our epistasis analysis revealed that *nhr-14/HNF4* deletion abrogated DNA damage-induced apoptosis in *akt-1(ok525)* (Figure 2A) and decreased apoptosis in *ced-9(n1653)* background (Figure 2D). Knockdown of *nhr-14/HNF4* led to dramatically decreased germline apoptosis in *abl-1(ok171)* mutants (Figure 2B).

In addition, because *brc-1* is the BRCA1 homolog in *C.elegans* and functions in DNA double strand break (DSB) repair [13, 14] after ionizing gamma-irradiation, mutation of *brc-1/BRCA1* resulted in failing to repair the double strand break and induce apoptosis. We also found that *brc-1(tm1145);nhr-14(tm1473)* double mutant exhibited dramatic decreased apoptosis compared to *brc-1(tm1145)* alone after DNA damage (Figure 2C). Taken together, these findings indicate that *nhr-14/HNF4* is a key regulator of DNA damage-induced programmed cell death.

nhr-14/HNF4 doesn't affect physiological programmed cell death

Since *nhr-14(tm1473)* showed less apoptosis upon gamma-irradiation, we next investigated underlying cellular mechanism. We performed the time lapse phenotype analysis and found that there was no germline development defect and *nhr-14(tm1473)* showed the same apoptosis number as N2 at any time. These data indicate the decreased programmed cell death in *nhr-14(tm1473)* is neither due to germline development nor the delayed cell death. We further examined whether *nhr-14* affects the physiological programmed cell death in embryo. Figure 3A shows that there was no difference in the number of cell apoptosis in embryo between N2 and *nhr-14(tm1473)*. *ced-1(e1735)* [15] and *vps-18(tm1125)* [16] has been reported to affect cell corpse clearance, we also found no difference in the number of cell apoptosis in germline between wild type and *nhr-14(tm1473)* mutant in the background of *ced-1(e1735)* [16] and *vps-18(tm1125)* [17] (Figure 3B, 3C). These results indicate that *nhr-14/HNF4* only affects the DNA damage-induced apoptosis, but not the physiological programmed cell death.

nhr-14/HNF4 functions downstream of the checkpoint pathway

Previous studies demonstrated that the checkpoint signaling pathways are activated upon DNA damage and play the critical role in repairing the damaged DNA or

inducing programmed cell death [17, 18]. Mutations in checkpoint genes can restrain both DNA damage-induced cell cycle arrest and apoptosis upon gamma-irradiation in *C. elegans* [17]. To determine whether *nhr-14*/HNF4 is a checkpoint gene, we first assessed the sensitivity of *nhr-14(tm1473)* mutant to gamma-irradiation using radiation sensitivity assay. We found that the survival rate of *nhr-14(tm1473)* progeny was comparable to that of wild-type animals, but was much higher than that of checkpoint gene mutant *hus-1(op244)* and *clk-2(mn159)* (Table 1). In addition, *nhr-14(tm1473)* worms displayed similar cell cycle arrest in germline mitotic region to that

in wild type following irradiation-treatment (Figure 4A). We further made *hus-1(op244); nhr-14(tm1473)* and *clk-2(mn159); nhr-14(tm1473)* double mutants, and found that these double mutants exhibited the same phenotype as the checkpoint mutants (Figure 4B). Therefore, *nhr-14*/HNF4 is not a checkpoint gene and may function downstream of the checkpoint pathway.

To determine whether *nhr-14*/HNF4 is involved in DNA repair, we irradiated worms containing the *hus-1::gfp* transgene in *nhr-14(tm1473)* background. We found that relocalization of HUS-1::GFP was independent of *nhr-14*/HNF4 (Supplementary Figure 1A), and the

Figure 1: Inactivation of *nhr-14*/HNF4 inhibits DNA damage-induced programmed cell death in *prmt-5(gk357)*. (A) Quantitative analysis of germ cell apoptosis in control RNAi- and *nhr-14* RNAi-treated N2 and *prmt-5(gk357)* animals. N2 and *prmt-5(gk357)* were fed with control RNAi and *nhr-14* RNAi and then (L4) was irradiated. After 36 hours of gamma-irradiation, germ cell apoptosis from one gonad arm of each animal were scored from at least 20 animals. Error bars represent standard error of the mean (SEM). ** and *** indicate $p < 0.01$ and 0.001 , respectively. (B) Quantitative analysis germ cell apoptosis induced by gamma-irradiation in N2, *nhr-14(tm1473)*, *prmt-5(gk357)* and *prmt-5(gk357); nhr-14(tm1473)*. Germ cell apoptosis from one gonad arm of each animal were scored after 36 h of irradiation at indicated doses. At least 20 worms were scored at each radiation dose or time point. Error bars represent standard error of the mean (SEM). (C) Quantitative analysis germ cell apoptosis at indicated time points after irradiation (120 Gy) in N2, *nhr-14(tm1473)*, *prmt-5(gk357)* and *prmt-5(gk357); nhr-14(tm1473)* animals.

number of foci in *nhr-14(tm1473)* was the same as wild type N2 (Supplementary Figure 1B). *nhr-14(tm1473)* is defective in irradiation induced apoptosis but is wild type for irradiation induced cell cycle arrest (Figure 4) and DNA repair. These findings suggest that *nhr-14/HNF4* is not involved in DNA repair and acts downstream of the checkpoint genes.

NHR-14/HNF4 cooperates with CEP-1/p53 to regulate *egl-1* and *ced-13* transcription upon DNA damage

To investigate how *nhr-14/HNF4* regulates DNA damage-induced programmed cell death, we first examined the expression level of apoptotic initiator gene *egl-1* and *ced-13* in N2 and *nhr-14(tm1473)* worms. We irradiated N2 and *nhr-14(tm1473)* young adult worms at a dose of 120 Gy and performed RT-qPCR experiment, our results show that gamma-irradiation induced *egl-1* and *ced-13* levels were significantly reduced in *nhr-14(tm1473)*. In N2

worms, *egl-1* level was increased by 20 folds, however, in *nhr-14(tm1473)* *egl-1* expression only increased 8 folds after DNA damage (Figure 5A). *ced-13* expression level was induced more than 5 fold in N2 worms upon gamma-irradiation, but only about 3 fold in *nhr-14(tm1473)* worms (Figure 5B). These results suggest that *nhr-14/HNF4* regulates DNA damage-induced *egl-1* and *ced-13*.

Previous studies demonstrated that CEP-1/p53 is a key transcription factor of *egl-1* and *ced-13* [18, 19]. Because *nhr-14/HNF4* regulates *egl-1* and *ced-13* at mRNA level, we hypothesized that NHR-14/HNF4 could be a cofactor of CEP-1/p53. To this end, we first examined if NHR-14/HNF4 forms a complex with CEP-1/p53. Flag-tagged NHR-14 and Myc-CEP-1 were co-transfected into 293T cells, and then CoIP was performed. Figure 5C shows that Myc-CEP-1/p53 was co-immunoprecipitated with Flag-NHR-14, suggesting that these two proteins interact each other in mammalian cells. To investigate if NHR-14/HNF4 directly binds to CEP-1/p53, we performed *in vitro* GST-pull down assay. We found that

Figure 2: Epistasis analysis indicates *nhr-14/HNF4* mediating DNA damage-induced apoptosis. (A) Quantitative analysis germ cell apoptosis induced by gamma-irradiation in N2, *nhr-14(tm1473)*, *akt-1(ok525)* and *akt-1(ok525); nhr-14(tm1473)*. Young adult animals were irradiated with gamma-ray (120 Gy) and analyzed at indicated time points after irradiation. Error bars represent standard error of the mean (SEM). (B) Quantitative analysis germ cell apoptosis in control RNAi and *nhr-14* RNAi-treated N2 and *abl-1(ok171)* animals. N2 and *abl-1(ok171)* were fed with control RNAi and *nhr-14* RNAi and then (L4) was irradiated at 120Gy. After 36 hours of gamma-irradiation, germ cell apoptosis from one gonad arm of each animal were scored from at least 20 animals. Error bars represent standard error of the mean (SEM). (C) Quantitative analysis of germ cell apoptosis induced by gamma-irradiation in N2, *nhr-14(tm1473)*, *brc-1(tm1145)* and *brc-1(tm1145); nhr-14(tm1473)* animals. (D) Quantitative analysis germ cell apoptosis induced by gamma-irradiation in N2, *nhr-14(tm1473)*, *ced-9(n1653)* and *ced-9(n1653); nhr-14(tm1473)* animals.

GST-CEP-1 fusion protein, but not GST, pulled-down ^[35S]methionine labeled NHR-14 (Figure 5D). These data suggests that NHR-14/HNF4 and CEP-1/p53 might directly interact each other.

We next investigated whether *nhr-14* /HNF4 regulates DNA damage induced programmed cell death through *cep-1*/p53. As GLD-1 is a translational repressor of *cep-1*/p53 via directly binding to the 3'UTR of *cep-1*/p53 mRNA [20], *gld-1*(*op236*) loss of function mutant expresses higher level CEP-1/p53 in *C.elegans*. We found that *egl-1* and *ced-13* mRNA level were much higher in *gld-1*(*op236*) mutant than N2 worms after gamma-irradiation (Figure 5A, 5B). We also demonstrated that up-regulated CEP-1/p53 in *gld-1*(*op236*) could rescue DNA damage-induced *egl-1* and *ced-13* expression and apoptosis (Figure 5A, 5B and 5E).

In conclusion, our data suggest that *nhr-14*/HNF4 functions as a cofactor of *cep-1*/p53 and regulates DNA damage-induced programmed cell death through CEP-1/p53 (Figure 5F).

DISCUSSION

DNA damage-induced programmed cell death is associated with various human malignancies and identification of regulators in DNA damage-induced apoptosis pathway is critical for intervention of these diseases. *C.elegans* has been shown to be an excellent model to study DNA damage-induced programmed cell death. And thus it is very helpful for us to understanding the mechanism of carcinogenesis by studying the regulation of DNA damage-induced apoptosis in *C.elegans* germline.

Figure 3: *nhr-14* doesn't affect the physiological programmed cell death. (A) Quantification of embryo cell apoptosis in N2 and *nhr-14*(*tm1473*) mutants. (B) Quantification of germline cell apoptosis in *ced-1*(*e1735*) and *ced-1*(*e1735*); *nhr-14*(*tm1473*) animals at indicated time points post L4. (C) Quantification of germline cell apoptosis in *vps-18*(*tm1125*) and *vps-18*(*tm1125*); *nhr-14*(*tm1473*) animals at indicated time points post L4.

Table 1: *nhr-14* doesn't affect the survival of progeny after gamma-irradiation treatment the survival of *nhr-14(tm1473)* mutant progeny is not sensitive to irradiation

irradiation		Survival(%)				
Dose(Gy)	<i>N2</i>	<i>nhr-14(tm1473)</i>	<i>hus-1(op244)</i>	<i>hus-1;nhr-14</i>	<i>clk-2(mn159)</i>	<i>clk-2;nhr-14</i>
0	100.0±0	100.0±0	97.6±0.1	98.3±0.8	98.0±0.7	96.0±1.5
40	86.3±1.2	86.1±1.8	37.7±1.1	39.6±2.0	29.1±2.6	28.9±1.7
80	76.7±2.4	76.3±1.8	18.7±1.3	18.0±1.2	9.4±0.5	11.1±0.8
120	64.5±1.6	62.5±1.4	3.2±0.5	3.5±0.7	2.8±1.0	2.8±1.0

P53 is a key tumor suppressor and its mutations were detected in more than 50% of human cancers. In *C.elegans*, the p53 homolog CEP-1 acts as a key effector to mediate germ cell apoptosis triggered by ionizing irradiation [21]. Identification of new co-factors of CEP-1/p53 in *C.elegans* may offer critical targets for cancer intervention.

In response to DNA damage stimuli, the checkpoint genes will sense the signals and induce cell cycle arrest or programmed cell death. Simultaneously, CEP-1/p53 is activated and subsequently induces up-regulation of BH3 genes *egl-1* and *ced-13*. Mutation of the checkpoint genes block the transfer of DNA damage signals and reduce DNA damage-induced apoptosis. Nuclear hormone receptor family is a key to many important cellular processes, but the role of NHR family in DNA damage-induced programmed cell death remains elusive. Previous study showed that NHR-14/HNF4, which was thought to be an estrogenic hormone receptor [8], was involved in the

immune response processes via regulation of vitellogenin expression [22]. In present report, we identified *nhr-14*/HNF4 as an important member of NHR in regulation of DNA damage-induced apoptosis. Moreover, we showed that *nhr-14*/HNF4 is primarily involved in regulation of the DNA damage-induced apoptosis, but not the physiological programmed cell death (Figure 3).

Mechanically, our experiment revealed that *nhr-14*/HNF4 deletion decreases DNA damage-induced up-regulation of *egl-1* and *ced-13*. More significantly, we showed that NHR-14/HNF4 might interacts with CEP-1/p53 and functions as a cofactor of CEP-1/p53. In addition, *nhr-14(tm1473)* mutant dramatically reduces CEP-1/p53-mediated DNA damage-induced apoptosis. Therefore, our study first reported a nuclear hormone receptor NHR-14/HNF4 that is involved in DNA damage-induced apoptosis. Identification of NHR-14/HNF4 interaction with CEP-1/p53 to control DNA damage-induced *egl-1* and *ced-13* could provide new targets for cancer intervention.

Figure 4: *nhr-14* is not a checkpoint gene and does not affect the cell cycle arrest after DNA damage. (A) Representative image of DAPI staining of the germline mitotic region in N2, *nhr-14(tm1473)* and *hus-1(op244)* worms. Young adult worms were treated with gamma-irradiation at 120 Gy. After 36 hours of irradiation, germline was dissected and stained with DAPI. Bars, 5 μ m. **(B)** Quantification of germline mitotic nuclear number after gamma-irradiation in N2, *nhr-14(tm1473)*, *hus-1(op244)*, *hus-1(op244);nhr-14(tm1473)*, *clk-2(mn159)*, *clk-2(mn159);nhr-14(tm1473)* worms. The gray and black bars represent nuclei number in mitotic region in control and gamma irradiation-treated worm germline, respectively. *** indicate $p < 0.001$.

Figure 5: NHR-14 cooperates with CEP-1/p53 to regulate *egl-1* and *ced-13* expression. (A) Relative fold induction of *egl-1* mRNA in N2, *nhr-14(tm1473)*, *gld-1(op236)* and *gld-1(op236);nhr-14(tm1473)* after 24 hours of gamma-irradiation (120 Gy). *egl-1* fold induction was averaged from three independent RT-qPCR analyses. (B) Relative fold induction of *ced-13* mRNA in N2, *nhr-14(tm1473)*, *gld-1(op236)* and *gld-1(op236);nhr-14(tm1473)* following 24 hours of gamma-irradiation (120 Gy). *egl-1* fold change was averaged from three independent RT-qPCR analyses. (C) NHR-14 interacts with CEP-1/p53 in mammalian cells. Flag-NHR-14 and Myc-CEP-1/p53 were co-expressed in HEK293 cells and then immunoprecipitated (IP) using Flag antibody. The immunoprecipitated proteins were detected by immuno-blotting (IB) with Myc antibody. (D) NHR-14 and CEP-1/p53 directly interact *in vitro*. The full-length NHR-14 protein was *in vitro* translated and labeled with [³⁵S] methionine and incubated with GST or GST-CEP-1/p53 fusion proteins, which were immobilized on glutathione sepharose beads, for 2 h. After extensive washes, the bound proteins were resolved by SDS-PAGE and detected by autoradiography (E) Quantitative analysis germ cell apoptosis induced by gamma-irradiation at indicated time points post L4 in N2, *nhr-14(tm1473)*, *gld-1(op236)* and *gld-1(op236);nhr-14(tm1473)*. Up-regulated CEP-1/p53 via loss function of *gld-1(op236)* rescued DNA damage-induced programmed cell death in *nhr-14(tm1473)* animals. (F) The genetic pathway for *nhr-14*/HNF4 to regulate DNA damage-induced apoptosis. NHR-14/HNF4 functions as a cofactor of CEP-1/p53 to regulate DNA damage induced apoptosis via controlling *egl-1* and *ced-13* expression.

MATERIALS AND METHODS

C. elegans strains and genetics

The strains of *nhr-14(tm1473)*, *brc-1(tm1145)* and *vps-18(tm1125)* were provided by Dr. Shohei Mitani. *prmt-5(gk357)*, *cep-1(gk138)*, *gld-1(op236)*, *akt-1(ok525)*, *abl-1(ok171)*, *ced-9(n1653)*, *hus-1(op244)* and *clk-2(mn159)* strains were provided by *C. elegans* Genetic Center (CGC). Worms were cultured and maintained using standard procedures. The Bristol N2 strain was used as wildtype. Deletion strains were outcrossed with N2 strain for 6 times. Double mutants were constructed with standard protocol.

Germ cell apoptosis assay

Synchronized young adult animals were irradiated with gamma-Ray (120Gy), which was located in the Peking University Health Science Center. Irradiated animals were put back to culture at 20°C at different time points. Worms with normal germline morphology were scored for germline cell apoptosis with DIC Zeiss microscope.

Radiation sensitivity assay

N2 worms and *nhr-14(tm1473)* and *hus-1(op244)*, *hus-1(op244);nhr-14(tm1473)*, *clk-2(mn159)*, *clk-2(mn159);nhr-14(tm1473)* mutant worms were irradiated at the L4 stage as indicated. Eggs laid 8–24hr after irradiation (corresponding to pachytene-stage germ cells at the time of irradiation) were counted. Unhatched eggs surviving animals were counted for days 1 and 2. The result represents the percent of survival of embryos of six different animals per strain.

Mammalian cell culture, transfection and immunoprecipitation

Human embryonic kidney (HEK293) cells were grown in Dulbecco's modified Eagle's medium (HyClone) supplemented with 10% fetal bovine serum (HyClone). The transfection was performed with 2.0 µg of mammalian vectors expressing worm proteins with different tags (i.e., pCMV-myc-*cep-1*, pCMV-tag2B-*nhr-14*) using PEI reagent. After 36h of transfection, cells were harvested and lysed in a buffer containing 50mM Tris (pH 8.0), 150mM NaCl, 0.5% sodium deoxycholate, 1% Triton X-100, 1mM phenylmethylsulfonyl fluoride (PMSF). The lysate was incubated with anti-Flag antibody (M2)-conjugated agarose beads (Sigma) for more than 2 h at 4°C. The beads were washed extensively in a buffer containing 50mM Tris (pH 8.0), 150mM NaCl, 1mM PMSF and 1% NP-40. Bound proteins were eluted and resolved on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and detected with Western blot assay.

Western blot assay

Cells were scraped and lysed in lysis buffer on ice for 15 min, 15 µg total proteins were loaded on SDS-PAGE Gel as co-immunoprecipitation experiment input. The SDS-PAGE gel first run on 60V for 30min and then 120 V until the dye run out of the gel, then the protein was transferred to PVDF membrane. The membranes were blocked in 5% nonfat dry milk in Tris-buffered saline, 0.05% Tween for 30 minutes at room temperature, and then incubated with primary antibodies for 2–4hours at 4°C, followed by incubation with secondary antibody for 60 min at RT. The primary antibodies used in this study were as follows:

anti-Flag (Sigma, Cat#:F3165) and anti-Myc (Sigma, Cat#:HPA055893).

GST pull-down assay

For GST pull-down assay, purified GST or GST-CEP-1 fusion proteins were immobilized on glutathione-Sepharose beads and incubated with [³⁵S]methionine-labeled NHR-14 at 4°C for more than 2 h. The beads were washed extensively and bound proteins were eluted and separated on 12% SDS-PAGE and exposed to phosphor-imager (Amersham) for autoradiography.

RT-qPCR assay

Total *C.elegans* RNA was extracted using TRIZOL method and cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad Laboratories). qPCR was performed in an iCycler thermocycler (Bio-Rad Laboratories) using iQ SYBR Green Supermix (Bio-Rad Laboratories). mRNA levels were quantified using iCycler software (Bio-Rad Laboratories) and were normalized to *tbg-1*. The primers used for RT-qPCR were as follows:

egl-1 q-PCR NS: 5'-gattcttcaattgcccagc-3'; *egl-1* q-PCR CAS: 5'-tcactgagcatcgaagtcac-3'; *ced-13* q-PCR NS: 5'-acggtgttgagttgcaagc-3';

ced-13 q-PCR CAS: 5'-gtcgtacaagcgtgatgat-3'; *tbg-1* q-PCR NS: 5'-cgctcatcagcctggtagaaca-3'; *tbg-1* q-PCR CAS: 5'-tgatgactgtccagttgga-3'.

All experiments were analyzed in triplicates.

ACKNOWLEDGMENTS AND FUNDING

We thank *C. elegans* Genetic Center and Dr. Shohei Mitani for providing *C.elegans* deletion strains. We especially thank Dr. Chonglin Yang for helpful suggestion and reagents. We also thank Dr. Shengyu Yang for comments on the manuscript.

This work was supported by National Natural Science Foundation of China (31671448 and 81472264), Natural Science Foundation of Shandong Province (ZR 2015CL034) and Advanced Talents Foundation of Qingdao Agricultural University (663/1113307).

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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