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Evidence of communication between heat-stressed and unheat-stressed *Caenorhabditis elegans* via volatile stress signal

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ABSTRACT

Our research group has recently described that radiation-induced airborne stress signals can be communicated between *Caenorhabditis elegans* (*C. elegans*). This paper addresses the question of whether heat stress can also induce emission of airborne stress signal to alert neighboring *C. elegans* and elicit their subsequent stress response. Here, we report that heat-stressed *C. elegans* produces volatile stress signal(s) that trigger an increase in radiation resistance in neighboring unheat-stressed *C. elegans*. When several loss-of-function mutations affecting the thermosensory neuron (AFD), heat shock factor-1 (HSF-1), and small heat-shock proteins (HSPs) were used as the heat-stressed *C. elegans,* we found that the production of the volatile stress signal(s) were blocked, demonstrating the heat shock response as a role in controlling the production of the volatile stress signal(s). It can be found that the mutations affecting DNA damage response (DDR) could inhibit the increasing of radiation resistance in neighboring unheat-stressed *C. elegans* having received the volatile stress signal(s), which indicate that the DDR might contribute to RAR induction by the volatile stress signal(s). Together, this study demonstrates that heat-stressed nematodes could communicate with unheat-stressed nematodes via volatile stress signal. In addition, the regulating pattern of signal production and action are preliminarily clarified.

Keywords: chemical communication, volatile stress signal, heat stress, *C. elegans*

1. INTRODUCTION

Current knowledge shows communication within and between organisms, i.e. bacteria, protozoa, animals, fungi and plants, as essential[1]. Compared to biocommunication of bacteria, fungi, plants and viruses, animals can not only identify group identity of self and nonself relying on volatile substances such as pheromones, but also transport various meaning via tactile behavior, vocal sounds and visual gestures[1]. Since allelopathic effects are easily confused by behavioral and instinctive responses, it is difficult to prove whether chemical stress or warning signal can be transmitted in the animal world[2]. It has been reported that when faced with stress of high density or food shortage, *Daphnia* species can suppress reproduction of individuals in order to protect the survival of the entire population by sensing chemical stress signals of high density and food shortage at a population level[3]. In mammals, Surinov et al. showed that irradiated mice could generate stress signals and transmit them to the unirradiated mice[4]. Recent researches have further demonstrated the communication of radiation-induced stress signals in rainbow trout (*Oncorhynchus mykiss, W*), zebrafish (*Danio rerio*), Zebrafish Embryos, and *Caenorhabditis elegans* (*C.elegans*)[2, 5-7]. It is of great significance for the survival of the population to explore the stress signals communicated in vivo between organisms.

The nematode *C. elegans* has emerged as an important model animal and been widely used to investigate the innate immune system, signal transduction, development, and nervous system, mainly due to the features of easy maintenance, the short life span of the organism for approximately 15-21 days, small body size, the

abundance of mutant strains, and the fact that results of trials on *C. elegans* can be predictive of outcomes in higher organisms[8-12]. Of the 959 somatic cells of the hermaphrodite some 300 are neurons including 12 pairs of amphid chemosensory neurons which mediate the reaction of detecting various water-soluble and volatile chemicals in the environment[8, 13-14]. Because of the absence of audition and vision, Inter-organismal communication relies on chemosensory neurons inputs in *C. elegans*. It has been reported that in times of high population densities stress, *C. elegans* sensed ascarosides by several types of chemosensory head neurons to induce development of the dauer larval stage at high population densities, as well as to control various behaviors, including sexual attraction[15-21], avoidance, aggregation[20, 22-23], olfactory plasticity[24-25], lifespan[26], and stress resistance[26-27]. Recent research by Yu peng et al. has showed that irradiated *C. elegans* can communicate stress signals with unirradiated *C.elegans* by the cysteine protease CPR-4 secreted from animals irradiated with UVC or gamma rays[7]. In addition to these water-soluble cues, *C. elegans* relies on their olfactory sensory neurons to sense a large number of volatile compounds, such as natural products of bacterial metabolism[27-30].

Recently our group has demonstrated that *C. elegans* at 25Gy irradiated with gamma-irradiation released volatile stress signal to induce radio-adaptive responses (RAR) in naive (unirradiated) *C. elegans*[31]. However, so far there have been few reports about volatile signal between *C. elegans* under natural stress. In order to determine whether *C. elegans* produce and response to such a type of volatile stress signal, we used previous co-cultivation system[31], in which radio-adaptive responses

(RAR) of embryonic lethality was used as a physiological/developmental endpoint to evaluate the presence of volatile stress signal.

2. RESULTS

2.1 Heat-stress-induced production of the volatile stress signal(s) in C. elegans

In order to confirm the existence of volatile stress signals released by heat-stressed *C. elegans*, we used a co-culture experimental system (Fig. 7), in which two Petri dishes ("top" and "bottom") were sealed together with a parafilm. The embryonic lethality of the top worms was used as a cue to detect volatile stress signal. Firstly, the bottom worms were heated at 35 ℃ for 0-2h, and were then co-cultured with the top worms for 6 h. The embryonic lethality in naïve top worms (0 Gy) was examined and not affected (in all cases, $P > 0.05$), as shown in Fig. 1A. Our group has recently demonstrated that *C. elegans* irradiated with gamma-irradiation at 25Gy released volatile stress signal to induce radio-adaptive responses (RAR) in naive (unirradiated) *C. elegans*[31]. We then hypothesized that the heat-stress-induced volatile signal might induce RAR in the top worms. In order to verify this possibility, we chose 25Gy as the challenge doses after co-cultivation of the top worms for 6 hours. As shown in Fig. 1A, interestingly, heat exposure of bottom worms for 20 mins, 40 mins, 60 mins, or 90 mins significantly reduced embryonic lethality in top worms irradiated with 25 Gy (in all cases, $P < 0.05$), we focued on the use of a combination of 1 h/35 ℃ (bottom) + 25 Gy(top) unless otherwise specified. *C. elegans* can be grown at temperatures ranging from 12 ℃ to 25 ℃, and will be subjected to heat stress once above 25 ℃ [32]. To verify that different heat stress can induce the production of volatile signal, the bottom worms were heated at 20/25/30/35 ℃ for 1h. The results show that the heat exposure of the bottom worms at 30/35 ℃

significantly reduced embryonic lethality in the top worms irradiated with 25 Gy (in all cases, $P < 0.05$), while the growth of the bottom worms at normal temperature did not affect the embryonic lethality of the top worms (in all cases, $P > 0.05$), as shown in SFig. 1. It has been reported that *C. elegans* is able to find food by sensing the smell of bacterial metabolic[33]. To eliminate the possibility that the volatile signal comes from *E. coli* OP50 under heat stress, *E. coli* OP50 on bottom dishes were heated with 35 ℃ alone and had no effects on the embryonic lethality of top worms (SFig. 2). *C. elegans* can sense various volatile chemicals via the olfactory nerve[8], the embryonic lethality was not changed when sensory nerve function was absent (*che-2, str-2, odr-3, and odr-1*) in the top worms (in all cases, $P > 0.05$), as shown in Fig. 1B. The above results demonstrated that heat-stressed worms could produce volatile stress signal(s) which initiated RAR in neighboring nematodes.

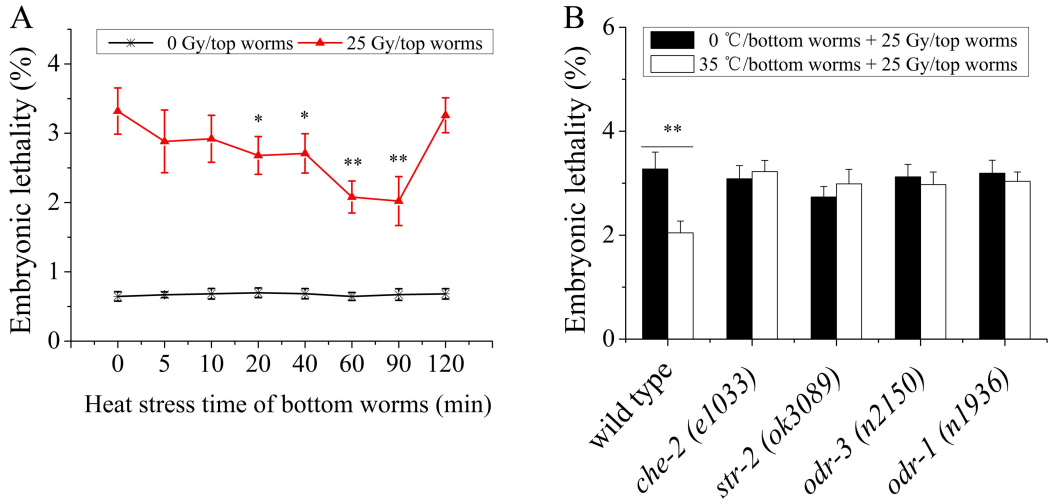

Figure 1. Heat-stressed bottom worms can induce RAR of embryonic lethality in top worms via the volatile stress signal(s). A) The change of embryo lethality of top worms after co-culture with bottom worms subjected to 35℃ heat exposure; B) Embryonic lethality of

top worms whose sensory nerve function is absent (*che-2, str-2, odr-3, and odr-1*) after co-culture with the bottom worms (N2). Results are means ± SD (n = 5, * *P* < 0.05, ** *P* < 0.01).

2.2 Effects of nematode development stages and culture density on production of the stress volatile signal(s) induced by heat stress

The life cycle of *C. elegans* is comprised of the embryonic stage, four larval stages (L1-L2-L3-L4), young adult and adulthood, some chemical signals secreted by *C. elegans* are closely related to its developmental stages and density[34]. Therefore, we first detected the effect of developmental stages of the bottom worms on production of the volatile stress signal(s). As shown in Fig. 2A, only heat-stressed L1 worms didn't alleviate embryonic lethality (in all cases, $P > 0.05$) and heat-stressed worms during other developmental stages could reduce embryonic lethality in the top worms (in all cases, $P < 0.01$). Furthermore, we examined the effect of culture density of the bottom worms on production of the volatile stress signal(s). As shown in Fig. 2B, the RAR of embryonic lethality was induced in the top worms when the culture density of the bottom worms at 200, 400, and 800 worms per dish (wpd) (in all cases, $P < 0.01$), but the RAR of embryonic lethality was prevented when the culture density of the bottom worms at 100 wpd ($P > 0.05$). Therefore, the stage and density of the bottom worms were L3 stage and 400 wpd, respectively, in the following experiments unless otherwise specified.

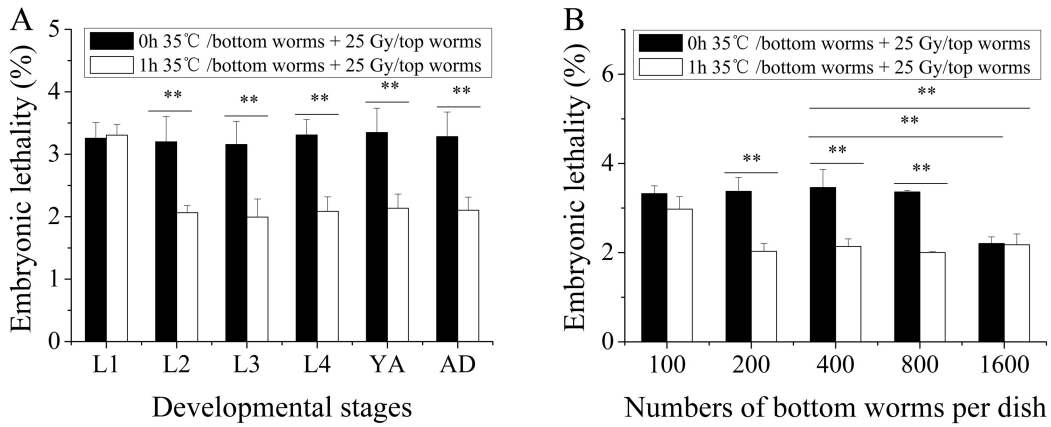

Figure 2 The production of the volatile stress signal(s) in heat-stressed worms depends on their developmental stage and culturing density. A) Effect of the developmental stage on the production of the volatile stress signal(s); B) Effect of the culturing density on the production of the volatile stress signal(s). Results are means ± SD (n = 5, ** $P < 0.01$).

2.3 Time course of production of the volatile stress signal(s) induced by heat stress in the bottom worms

In order to determine the time course of the production of the volatile stress signal(s) induced by heat stress in the bottom worms, the bottom worms were removed from the co-culture system after 2 h, 4 h, and 6 h. The embryonic lethality of the top worms was alleviated only after 6 h of co-culture with the heat-stressed bottom worms (in all cases, $P < 0.05$), as shown in Fig. 3A, indicating that it took about 6 h for bottom worms to produce enough volatile stress signal(s) for RAR induction after heat stress. The bottom worms were transferred into the co-culture system at 0 h, 2 h, and 4 h after heat stress respectively, and then the top worms were exposed to 25Gy at the 6th hour. It was shown that the RAR of embryonic lethality in the top worms only occurred when move-in at 0 h after heat stress of the bottom

worms ($P < 0.05$) (Fig. 3B), suggesting that the volatile stress signal(s) were produced immediately after heat stress in the bottom worms.

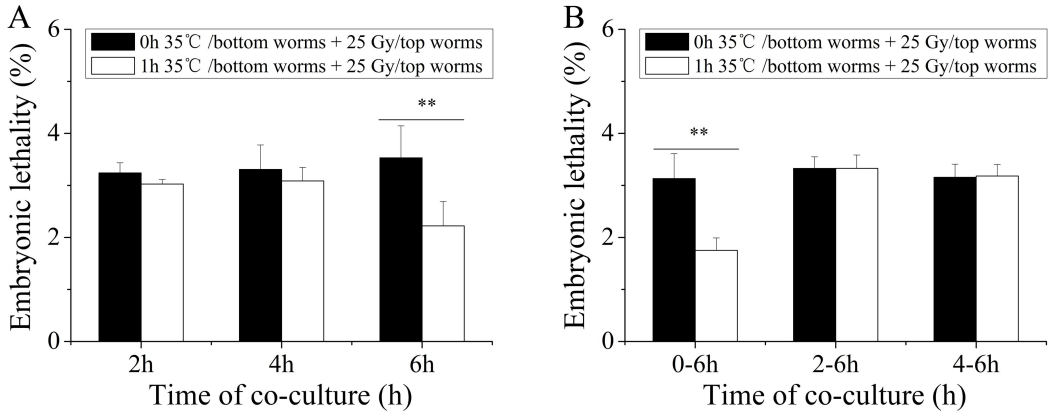

Figure 3 The induction of RAR in the top worms depends on the co-culture time of the bottom heat-stressed worms. A) Embryonic lethality of the top worms removed from the co-culture system at the designated time point after co-culture; B) Embryonic lethality of the top worms moved into the co-culture system at the designated time point after the heat shock of bottom worms. Results are means ± SD (n = 5, ** $P < 0.01$).

2.4. Effect of the stress volatile signal(s) on germ cells of the top worms

The hermaphrodite has both spermatheca and uterus, generating progeny primarily through self-fertilization[35]. Our results showed that heat-stress-induced volatile signal(s) could cause the top worms to initiate RAR to alleviate the embryonic lethality, which raised the question: whether RAR induced by the volatile stress signal(s) occurs in sperm cells or in female germ cells. To determine this, cell-death-defective *ced-3* and *ced-4* worms[36] were used as the top worms, considering that germ cell apoptosis occurs only during oocyte production in adult

hermaphrodites[37]. The RAR induction in the top worms was apparently prevented by the absence of *ced-4* and *ced-3* (in both cases, $P > 0.05$), as shown in Fig. 4A, suggesting that the volatile stress signal(s) might primarily affect the female germ cells of the top worms. In nematodes, moreover, embryogenesis needs to go through the following stages: the fertilization stage, the full grown oocyte stage, the late pachytene stage, the pachytene nuclei stage, and mitotic stage, which has a fixed developmental time[38]. In order to examine which stage of the female germ cells is specifically affected by the volatile stress signal(s), the embryonic lethality of the top worms was detected at different time points after irradiation. As shown in Fig. 4B, the embryonic lethality of the top worms was reduced for 0 - 12 h, 0 - 28 h, 0 - 40 h, and 0 - 100 h after challenge irradiation, but did not affect the embryonic lethality of the top worms for 0-8 h after irradiation. Existing oocytes were completely exhausted within ~8 h after eggs production[38] (here, ~8 h after challenge irradiation), indicating that the volatile stress signal(s) might mainly act on the meiotic and mitotic proliferating zone of gonad.

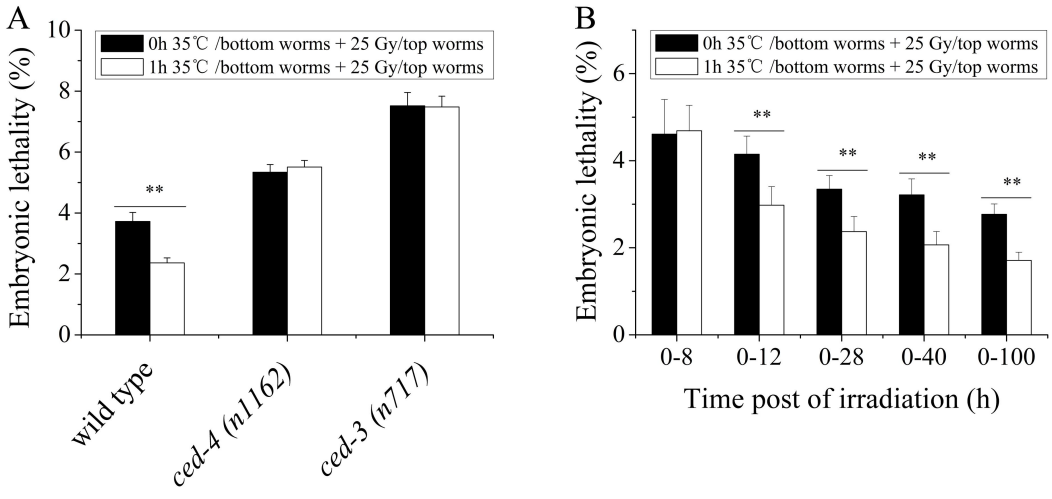

Figure 4 The volatile stress signal(s) mainly acts on the mitotic proliferating cell and meiosis cell of the gonads of top worms. A) Embryonic lethality of the top worms whose apoptosis function is absent (*ced-3* and *ced-4*) after co-culture with the heat-stressed worms (N2); B) Embryonic lethality of the top worms at different time points following challenge irradiation after co-culture with the heat-stressed worms. Results are means ± SD (n = 5, ** $P < 0.01$).

2.5. Role of DNA damage response (DDR) in the induction of RAR by the volatile stress signal(s)

The integrity of the genome is critical to the health of individual and the continuation of species. To maintain the fidelity of the genome, damaged genomes need to be monitored and repaired in organisms[39]. In response to ionizing radiation, *C. elegans* initiates DDR that induces checkpoint pathway, DNA repair, cell cycle arrest, or apoptosis to remove genetically damaged cells that might harm the organism[40]. Therefore, we raised the question whether DDR plays an important role in the RAR induction via the volatile stress signal(s). To illustrate this issue, several mutant worms that lost function in checkpoint pathway and DNA repair were used as the top worms. For the DNA damage checkpoints, the embryonic lethality of the mutant worms (for *atm-1*, *mrt-2*, and *cep-1* genes, $P > 0.05$; for the *hus-1* gene, $P < 0.01$) was not reduced except the mutant worms (for *clk-2* gene ($P < 0.01$), as shown in Fig. 5A, indicating DNA damage checkpoints play a key role in the induction of RAR by the volatile stress signal(s). The DNA repair pathways were located downstream of the DNA damage checkpoints, which whether also plays an important role in the induction of RAR will be discussed following. As shown in Fig. 5B, RAR induction

was prevented in the nucleotide excision repair (NER) pathway mutants (*xpf-1*), the non-homologous end joining (NHEJ) pathway mutants (*lig*-4 and *ku8*0), and the mismatch repair (MMR) pathways mutants (*msh-6*) (for the *xpf-1*, *lig*-4, and *msh-6* genes, $P > 0.05$; an increased embryonic lethality for the *cku-80* gene, $P < 0.05$), whereas RAR induction still existed in the homologous recombination (HR) pathways mutants (*brc-1*). These results indicate that the DNA damage checkpoints, NER, NHEJ, and MMR might contribute to RAR induction by the volatile stress signal(s).

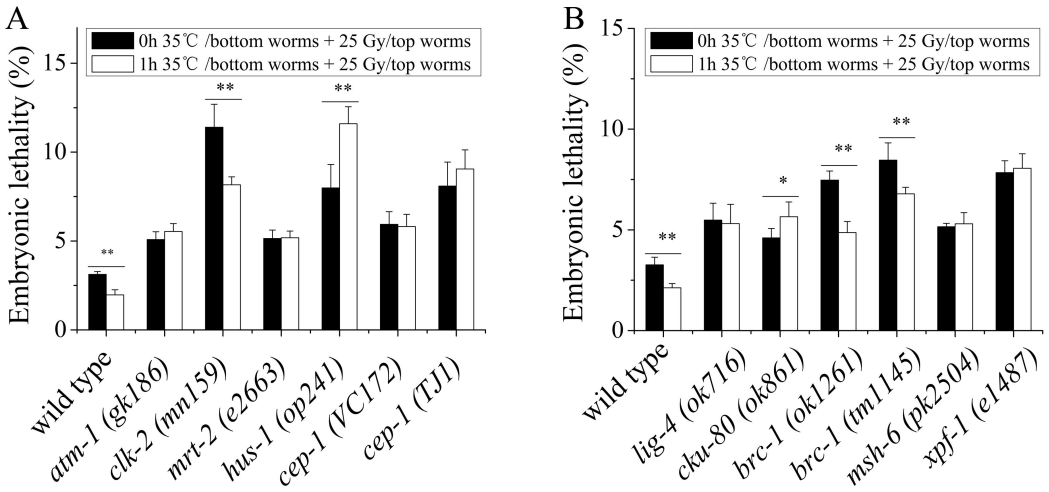

Figure 5 DDR is involved in the regulation of RAR induced by thve latile stress signal(s). A) Embryonic lethality of the top worms whose DNA damage checkpoint function (*atm-1, clk-2, mrt-2, hus-1, and cep-1*) is absent after co-culture with the heat-stressed worms (N2) ; B) Embryonic lethality of the top worms whose DNA repair function (HR, NHEJ , MMR, and NER) is absent after co-culture with the heat-stressed worms (N2). Results are means ± SD (n = 5, * $P < 0.05$, ** $P < 0.01$).

2.6. Regulation of heat shock response to production of the volatile stress signal(s)

In *C. elegans*, ascarosides are a prominent group of chemical signals that

mediates communication between worms of the same species, including male attraction, hermaphrodite repulsion, olfactory plasticity, and aggregation[15-21]. Joo et al. showed that the temperature affected biosynthesis of ascarosides[41]. Therefore, to check whether ascarosides could contribute to the production of the volatile stress signal(s) induced by heat stress in the bottom worms, mutant worms whose ascaroside biosynthesis function (*acox-1*, *maoc-1*, *dhs-2*8, and *daf-22* genes) is absent were used as the bottom worms in co-culture system. We found that heat-stressed ascaroside biosynthesis mutants (*acox-1*, *maoc-1*, *dhs-2*8, and *daf-22* genes) still induced the RAR of embryonic lethality in the top worms (in all cases, $P < 0.01$) (SFig. 3), suggesting that the production of the volatile stress signal(s) is not related to the ascaroside biosynthesis pathway. In order to cope with the rapid rise in temperature, all cells initiate a heat-shock response to increase thermal tolerance, prevent thermal tolerance, and reestablish cellular homeostasis[42]. Prahlad et al. showed that within *C. elegans*, the heat-shock response is not cell-autonomous but rather depends on the thermosensory neuron (AFD), which senses the surrounding temperature, thereby activating heat shock factor-1 (HSF-1) into the nucleus to control the expression of small heat-shock proteins (HSPs)[42]. Therefore, we tested whether the heat-shock response regulated the production of the volatile stress signal(s) induced by heat stress in the bottom worms. For this, several loss-of-function mutations affecting the AFD neuron, HSF-1, and HSPs were used as the bottom groups. As shown in Fig. 6A 6B 6C. *gcy-23* (thermosensory gene), *gcy-8* (thermosensory gene), *hsf-1*, *hsp-90*, *hsp-16.48*, *hsp-12.6*, *hsp-16.2*, and *hsp-4* genes all inhibited RAR induction in top

worms (in all cases, $P > 0.05$), suggesting that the heat-shock response have a role in controlling the production of the volatile stress signal(s); only *ocr-2* (the mutation does not affect the thermosensory function of the AFD neuron, but instead affects the sensory function of the four other neurons: ADF, AWA, ASH, and ADL) gene still presented RAR induction in the top worms (in all cases, $P < 0.05$), further indicating the heat shock response is essential in controlling the production of the volatile stress signal(s).

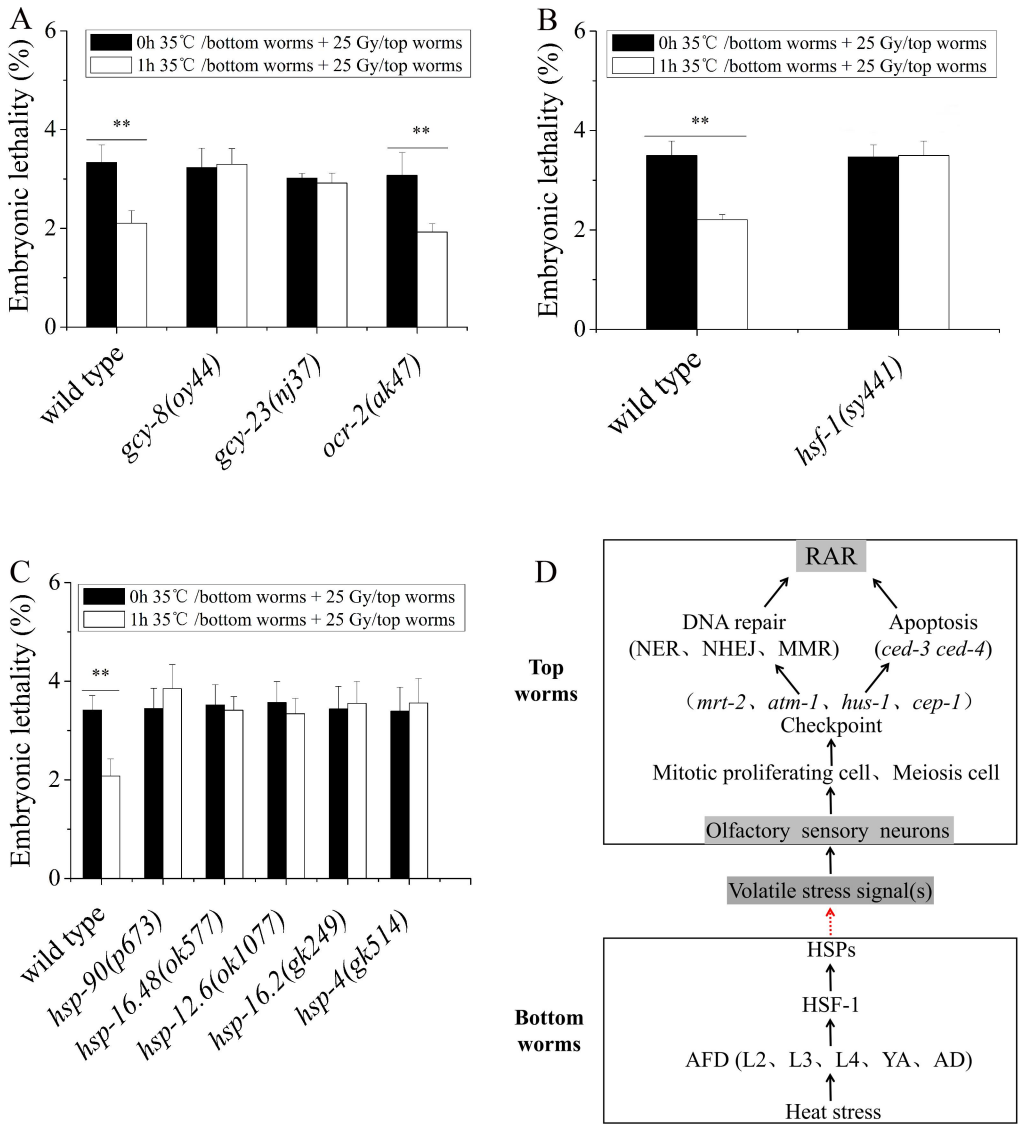

Figure 6 Heat-shock response regulates the production of the volatile stress signal(s) in the bottom worms under heat stress. A, B, C) Embryonic lethality of the top worms (N2) after co-culture with heat-stressed bottom worms whose thermosensory neuron (*gcy-8, gcy-23*, *and ocr-2*), *hsf-1,* and HSPs functions are absent; D) Proposed model of production and action of volatile stress signal(s). Bottom worms sense heat stress by AFD and initiate heat shock response. HSPs activate downstream related genes to produce volatile stress signal(s). Top worms sense volatile stress signal(s) by olfactory sensory neurons to activate DDR pathway (checkpoint, DNA repair, apoptosis) and induce RAR in meiotic and mitotic proliferating zone of gonad. Results are means ± SD (n = 5, ** *P < 0.01*).

3. Conclusion

To summarize, in this study, we demonstrate that nematodes are able to generate volatile stress signal(s) when subjected to heat stress. In order to prove that *C. elegans* has long-distance communication through volatile stress signal under heat stress, we have established a co-culture experimental system (Fig. 7). Firstly, we used the Petri dish coated with only *E. coli* OP50 exposed heat stress as the bottom dish in the co-culture system, and found that the production of the volatile stress signal(s) was inhibited (SFig. 2), which excluded the possibility that the volatile stress signal(s) were derived from heat-stressed *E. coli* OP50. Secondly, when several loss-of-function mutations affecting heat shock response and ascaroside biosynthesis were used as the bottom groups, the generation of the volatile stress signal(s) was blocked (Fig. 6A 6B 6C, SFig. 3), further indicating that the volatile stress signal(s) were not derived from heat-stressed *E. coli* OP50, but were produced by heat-stressed nematodes. Thirdly, the RAR was prevented when worms whose olfactory sensory neuron function (*che-2, str-2, odr-3, and odr-1*) is absent were used as the top groups (Fig. 1B). Since the top worms received airborne signals only through olfactory sensory neuron, this further suggested that heat-stressed nematodes could induce volatile stress signal. Finally, the RAR of the top worms could be induced only when the bottom worms were within the range of heat stress (SFig. 1). At the same time, we also found that the RAR could be induced in the top worms when the bottom worms were subjected to high density stress (16000 worms per dish) (Fig. 2B). The above results show that the worms in both heat stress and high density stress can produce

volatile stress signals. Heat stress and high density stress are the two most common types of stress in nature, which means that *C. elegans* is likely to produce volatile stress signals for communicating stress cues in a long distance when facing stress. Once an individual living organism is subjected to stress, the volatile stress signals will spread throughout the entire population, making the entire population stay a state of warning to decrease the damage.

Recently our group has demonstrated the production of radiation-induced volatile signal depends on ascarosides secreted by *C. elegans*[31]. In this study, we found that the production of volatile signals induced by heat stress is not related to ascarosides (Fig. S3), which means that the volatile signals were induced by heat stress and radiation stress that are most likely two different types of substances. When we used the heat shock response mutants as heat-stressed nematodes of the bottom, we found that the generation of the volatile stress signal(s) was blocked (Fig. 6A 6B 6C), indicating that the heat shock reaction was involved in the regulation of the volatile stress signal(s). When the *hsf-1* mutant was used as radiation-stressed worms of the bottom, the RAR was still induced in the top worms (Fig. S4), which further suggests that the volatile signals induced by radiation were different from that induced by heat stress. The heat shock proteins (HSPs) are not volatile and therefore they cannot be directly used as the volatile signal induced by heat stress. So how do the HSPs regulate the production of the volatile stress signal(s)? Initially, the HSPs are only molecular chaperones which function intracellularly in an ATP-dependent manner[43], but increasing evidence now suggests that the HSPs can not only be

355 secreted to vitro[44] but also directly activate the relevant downstream genes in cells[45].
356 Also they can be secreted in the extracellular and cell-associated compartments to
357 elicit a range of biological effects[46]. Here, to determine whether the HSPs can also be
358 secreted into the environment and sensed by nematodes to regulate the production of
359 the volatile stress signal(s), we used nematode with olfactory sensory neuron
360 impairments as the bottom worms and found the RAR did not disappear (Fig. S5),
361 indicating that heat-stressed nematodes did not secrete the HSPs to vitro. Therefore,
362 we speculate that there are two possible ways for heat-stressed nematodes to regulate
363 the production of the volatile stress signal(s) through HSPs: 1) HSPs directly activate
364 downstream signaling pathways to regulate the production of the volatile stress
365 signal(s); 2) HSPs bind to receptors on the surface of other cells in the body to initiate
366 related signaling pathways that regulate the production of the volatile stress
367 signal(s). Therefore, exploring how HSPs regulate the production of the volatile stress
368 signal(s) will be the focus of our next research work.

369 *p53* is a key regulatory of the DNA damage-induced checkpoint in mammals[47],
370 and is necessary to maintain gene stability and trigger apoptosis in abnormal cells that
371 may become tumor cells[48-50]. Our results show that *cep-1*, the *C. elegans* ortholog of
372 the human tumour suppressor *p53*[51], is necessary for top worms to induce RAR (Fig.
373 5A). *ced-3* and *ced-4* are essential core elements for apoptotic pathway in *C. elegans*[52].
374 CED-3 is a member of the caspase family of proteases; CED-4 is homologous to
375 mammalian Apaf-1 and is a positive regulator of CED-3[53]. Our results confirm that
376 the apoptotic pathway is involved in RAR induction by the volatile stress signal(s) in

the top worms (Fig. 4A). At present, most human cancers evade *p53* tumor suppressor activity by selecting for mutations in *p53* itself[54-55]. This suggests that the volatile stress signal(s) of heat stress are very likely to enhance the inhibition of cancer cells in mammals. Furthermore, our results indicate that the nucleotide excision repair (NER) pathway (*xpf-1*), non-homologous end joining (NHEJ)pathway (*lig-4* and *ku80*), and mismatch repair (MMR) pathway (*msh-6*) are involved in RAR induction by the volatile signal in the top worms (Fig. 5B). These genes (*xpf-1,lig-4, ku-80*, and *msh-6*) are not only homologous to hunman, but also have highly conserved in repair functions[39]. The volatile substance increases radiation resistance to nematodes, indicating that it may also have similar function in humans. According to reports, 60-80% of *C. elegans* genes have an ortholog in the human genome[56], which means that the substances secreted by nematodes themselves that induce radiation resistance are likely to have the same effect on humans. The chemical structure of the volatile signal(s) induced by heat stress have not yet been confirmed. Further analysis on its structure might provide a new way to search for anti-radiation drug.

Generally, here we canonically demonstrated heat-stressed nematodes could communicate with unheat-stressed nematodes via the volatile stress signal(s). While the regulating pattern of signal production and action are preliminarily clarified, as shown in Fig. 6D, its chemical nature is unclear, and this should be the primary topic of further investigation.

399 # 4. MATERIALS AND METHODS

400 ## *4.1. Worm strains and culture conditions*

401 All *C. elegans* strains were cultured at 20 ℃ using standard conditions[57], unless
402 otherwise noticed. The N2 Bristol strain was used as the wild-type strain. In addition,
403 the following mutant strains were used in the genetic analyses: VC1785:
404 *acox-1*(*ok2257*)I, VS18: *maoc-1*(*hj13*)II, VS8: *dhs-28*(*hj8*)X, and DR476:
405 *daf-22*(*m130*)II, MT2547: *ced-4*(*n1162*)III and MT1522: *ced-3*(*n717*)IV, VC381:
406 *atm-1*(*gk186*)I, SP506: *clk-2*(*mn159*)III, CB5348: *mrt-2*(*e2663*)III, WS2277:
407 *hus-1*(*op241*)I, VC172: *cep-1*(*gk138*)I, TJ1: *cep-1*(*gk138*)I, RB873: *lig-4*(*ok716*)III,
408 RB964: *cku-80*(*ok861*), RB1209: *brc-1*(*ok1261*)III, DW102: *brc-1*(*tm1145*)III,
409 NL2511: *msh-6*(*pk2504*)I, CB1487: *xpf-1*(*e1487*)II, CB1033: *che-2*(*e1033*)X,
410 VC2413: *str-2*(*ok3089*)V, CX2065: *odr-1*(*n1936*)X, CX2205: *odr-3*(*n2150*)V,
411 CB1377: *daf-6*(*e1377*)X, PR808: *osm-1*(*p808*)X, MT3762: *osm-3*(*n1540*)IV, PR802:
412 *osm-3*(*p802*)IV, and PR811: *osm-6*(*p811*)V, IK800: *gcy-8*(*oy44*) IV, IK427:
413 *gcy-23*(*nj37*) IV, CX4544: *ocr-2*(*ak47*) IV, PS3551: *hsf-1*(*sy441*) I, VC1099:
414 *hsp-4*(*gk514*) II, VC475: *hsp-16.2*(*gk249*) V, RB1098: *hsp-12.6*(*ok1077*) IV, RB791:
415 *hsp-16.48*(*ok577*) V, PR673: *hsp-90*(*p673*) V. All of the nematode strains were from
416 the *Caenorhabditis* Genetics Center (CGC).

417 ## *4.2. Heat stress treatment*

418 Worms were placed on 60 mm dishes containing NGM agar and bacteria at a
419 population density of 400 worms and at the L4 stage per plate. In order to avoid

dehydration, plates were sealed with parafilm and packed into closed carton boxes, and then put into the incubator that was running at 35°C.

4.3. Protocols for co-culture of worms

The plates with worms were transferred to the incubator at 20 °C immediately following heat stress treatment until the plates were returned to 20 ℃. The heat stressed worms were placed at the bottom of the co-culture system, and other operating methods were described in Tang et al.[31], as shown in Fig. 7. After 6 h of co-culture, the top worms were removed from the co-culture system, and then subjected to 25Gy. The irradiated worms were allowed to lay eggs for 36 h and then removed from the top Petri dishes. After 6 h, the number of unhatched eggs and hatched larvae (F1) on the top Petri dishes was counted under dissection microscope to calculate embryonic lethality[40].

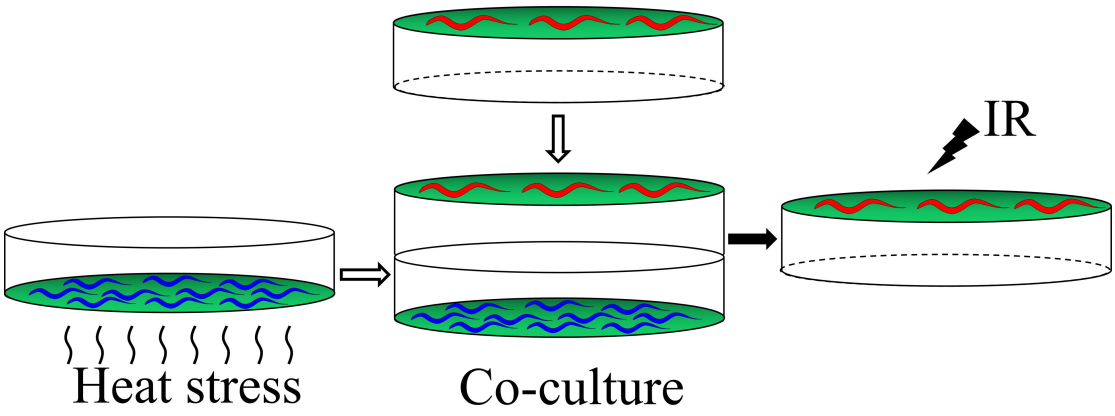

Figure 7. Schematic drawing of co-culture experimental system, in which red worms represent the top worms, blue worms represent the bottom worms, and the top worms communicate with the bottom via volatile signal(s).

4.4. Gamma-irradiation

The top worms were sealed with parafilm and exposed to gamma rays with 25Gy at a dose rate of 3.37 Gy/min using a Biobeam Cs137 irradiator (cat no. GM 2000; Gamma-Service Medical, Leipzig, Germany). The temperature of room must be kept at 20 °C when the worms were irradiated.

4.5. Statistical analysis

All experiments were repeated at least twice with identical or similar results. All results were presented as means ± standard deviations. All comparisons for differences among two and more than two data sets were determined by performing Student's *t-test*, with P values < 0.05 considered to be significant.

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Author contributions

L.C., Z.X., and P.B. designed research. L.C., T.W., Y.W., H.Z., F.W., H.L., and X.Z. performed research. L.C., T.W., and X.Z. analyzed data. L.C., Z.X., and P.B. wrote the paper.

Conflict of Interest statement

The authors declare that there are no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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