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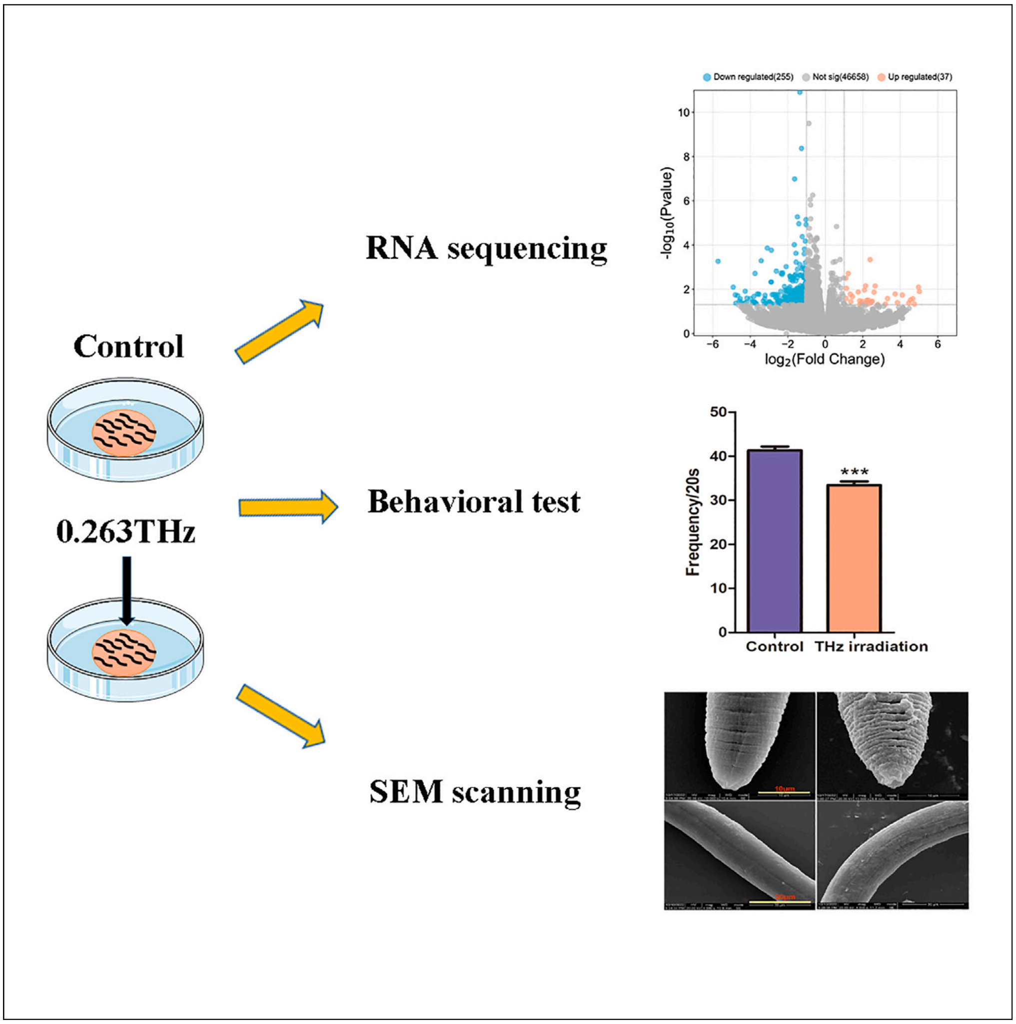

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Highlights

0.263 THz irradiation induced gene expression changes in *C.elegans*

0.263THz irradiation damaged the epidermal ultrastructures of *C. elegans*

0.263THz irradiation impaired the movement of *C. elegans*

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0.263 terahertz irradiation induced genes expression changes in *Caenorhabditis elegans*

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SUMMARY

The biosafety of terahertz (THz) waves has emerged as a new area of concern with the gradual application of terahertz radiation. Even though many studies have been conducted to investigate the influence of THz radiation on living organisms, the biological effects of terahertz waves have not yet been fully revealed. In this study, *Caenorhabditis elegans* (*C. elegans*) was used to evaluate the biological consequences of whole-body exposure to 0.263 THz irradiation. The integration of transcriptome sequencing and behavioral tests of *C. elegans* revealed that high-power THz irradiation damaged the epidermal ultrastructures, inhibited the expression of the cuticle collagen genes, and impaired the movement of *C. elegans*. Moreover, the genes involved in the immune system and the neural system were dramatically down-regulated by high-power THz irradiation. Our findings offer fresh perspectives on the biological impacts of high-power THz radiation that could cause epidermal damage and provoke a systemic response.

INTRODUCTION

In the past few decades, both fundamental and applied research fields have shown a great deal of interest in the electromagnetic radiation of the terahertz (THz) band, which corresponds to the wavelength range of 30 μm–3 mm with the frequency of 10^{11}–10^{13} Hz and lies between infrared and microwave frequencies.[1] As it matches the energy levels of biomolecular motion and interaction and has low photon energy, THz has been proven to have potential applications in the biomedical field, such as medicine, security, and imaging.[2–4] There have also been many recent studies focusing on the interaction of THz waves with neurons and the neural system, indicating the neuromodulatory potential of THz waves in living systems.[5–8]

However, it is crucial for us to note that although THz radiation is non-ionizing, several studies have discovered that exposure to THz radiation with certain power densities and durations could trigger a series of responses in various tissues and cells, resulting in potential biohazard effects. For example, a significant increase in neutrophil recruitment was observed after rats' ear skin was exposed to 2.7 THz radiation for 30 min.[9] Significant changes in the expression of many immune response-related genes were observed in human keratinocytes when exposed to three distinct frequencies: 1.4 THz, 2.52 THz, and 3.11 THz.[10] Even Kyu-Tae Kim et al. also found that femtosecond-terahertz (fs-THz) pulses perturb the wound healing process in mouse skin.[11] In addition to the skin, studies have shown that the neurological system can also be negatively affected by terahertz irradiation under certain conditions. Early research indicated that rats or mice exposed to THz may have higher levels of anxiety and sadness.[12,13] THz irradiation with an average power of 25 mW/cm^2 and 50 mW/cm^2 resulted in proliferative disorder, abnormal DNA damage repair, and increased apoptosis of neural stem cells.[14] The 0.12 THz wave with a power of 10 mW or the 0.157 THz wave with a power of 50 mW could also induce significant apoptosis in primary hippocampal neurons.[15] Hence, the alleged biosafety of THz does not necessarily mean there is no negative impact at all. More research is needed to understand the potential benefits or drawbacks associated with the exposure to THz waves.

In this study, we investigated the effects of radiation on *C. elegans*, a classic and well-established model organism for studying the bioeffects of ionizing and non-ionizing radiation through whole-body exposure.[16] Specifically, a single-frequency impulse wave laser source emitting at 0.263 THz and with a power density of 24 mW/cm^2 was employed to irradiate L4 stage *C. elegans* for 5 min. The transcriptome changes brought on by THz exposure were identified using transcriptome sequencing after irradiation. The bioinformatics analysis showed that terahertz irradiation had an impact on the expression of genes involved in the nervous system and immune system in *C. elegans*, offering fresh perspectives for further research into the biological impacts of THz.

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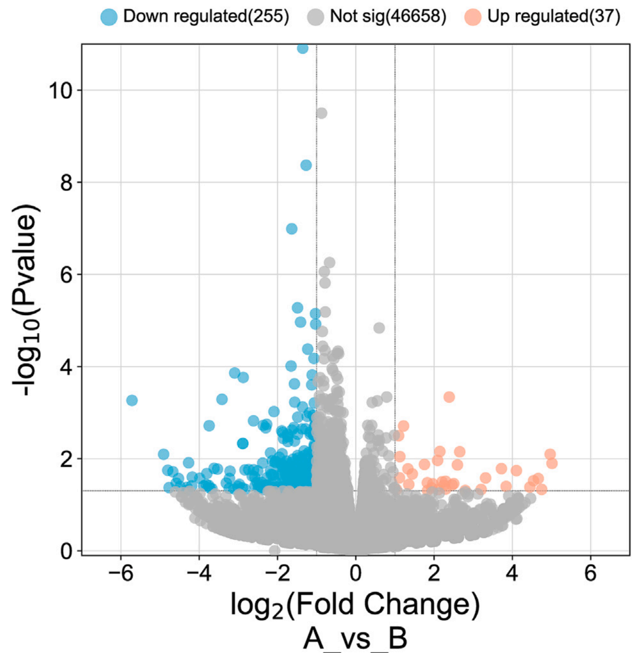

Figure 1. The different expression of genes induced by THz irradiation; The Volcano plot showing genes with differential expression in THz irradiation group compared to control group

RESULTS

The different expression of genes induced by THz irradiation

To investigate the genes affected by THz irradiation in C, the transcriptomes of mock and THz-irradiated *C. elegans* were analyzed using RNA-seq. Using the selection criteria (fold change $\geq$1.50 or $\leq$0.67 and a p value <0.05), high-throughput sequencing revealed 37 up-regulated differentially expressed genes (DEGs) and 255 down-regulated DEGs (Figure 1A). Thus 87.3% of the DEGs genes were down-regulated in response to THz irradiation. As a result, high-power terahertz irradiation is more likely to inhibit gene expression in *C. elegans.*

THz irradiation reduced the expression of cuticle collagen genes and induced altered cuticle structure

A gene ontology (GO) enrichment analysis of DEGs showed that the structural component of the cuticle was the most significant among the top 20 molecular functions (Figure 2A). Among these differentially expressed collagen genes, col-125, col-128, col-14, col-147, col-167, col-69, col-75, and col-89 were significantly down-regulated after terahertz irradiation, while only the expression of col-73 was up-regulated (Figure 2B). In order to determine if a pre-defined gene set displayed a significantly consistent up-regulation or down-regulation trend in the THz-irradiated group compared to the non-irradiated group, we also employed the gene set enrichment analysis (GSEA) method. The results demonstrated that the "construction of cuticle" was greatly enriched with a down-regulation tendency (Figure 2C). The THz-induced altered expression of the cuticle collagen genes resulted in changes to the cuticle structure, which were evident in our observation of the changes in the cuticle of *C. elegans* using scanning electron microscopy (SEM). As seen in Figure 2D, when compared to the control group, the THz irradiation group's entire body was heavily wrinkled from head to tail. The cuticle serves as a barrier and maintains the shape of the body. It is also a flexible structure that attaches to muscles to help with worm locomotion.[17] Therefore, the body bending ability of *C. elegans* was examined immediately after THz irradiation. As shown in Figure 2E, THz irradiation significantly reduced the movement of *C. elegans*, which might be attributed to the abnormal cuticle structure. These findings suggested that high-power THz irradiation may cause a cuticle lesion and impair the motor function of *C. elegans.*

THz irradiation reduced the expression of immune response genes

The GO analysis of the biological process indicated that the term "immune response" was considerably enriched in the top 20 biological processes with 12 genes involved. All 12 of these genes were down-regulated by THz irradiation (Figures 3A and 3B). Among these genes, irg-4, irg-5, dod-22, and dod-24, all of which contain a CUB-like domain, code for a collection of secreted proteins thought to be involved in host defense.[18] Meanwhile, six genes, abu-8, abu-10, abu-11, abu-13, pqn-32, and pqn-7, involved in the endoplasmic reticulum unfolded protein response were also enriched, and their expression was similarly decreased by THz irradiation (Figures 3A–3C). The GSEA analysis also revealed a down-regulation trend in the gene set of the endoplasmic reticulum unfolded protein response after THz irradiation (Figure 3D). In fact, these genes enriched in the endoplasmic reticulum unfolded protein response were also proven to play a crucial role in the innate

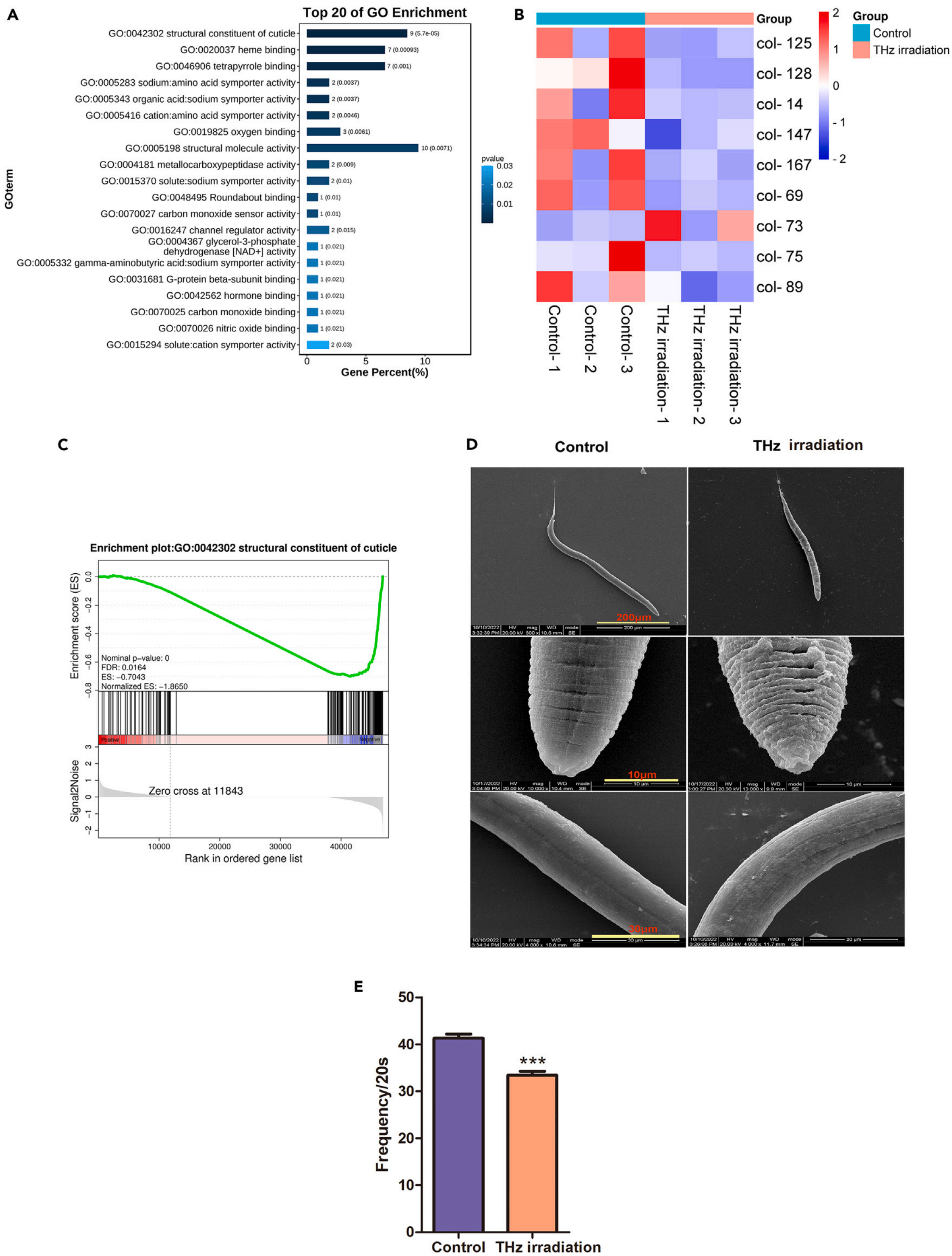

Figure 2. THz irradiation reduced the expression of cuticle genes and induced altered cuticle structure

(A) The functions of the different expression genes involved in Molecular Function analyzed by gene ontology analysis; (B) The clustering heatmap of 9 genes in structural constituent of cuticle; (C) The enrichment plot of structural constituent of cuticle gene set analyzed by GSEA analysis; (D) Surface of worms exposed or unexposed to THz irradiation; (E) The locomotion of *C.elegans* were examined by body bends within 20s; Data are shown as mean ± SEM, ***$p < 0.001$, compared with control group.

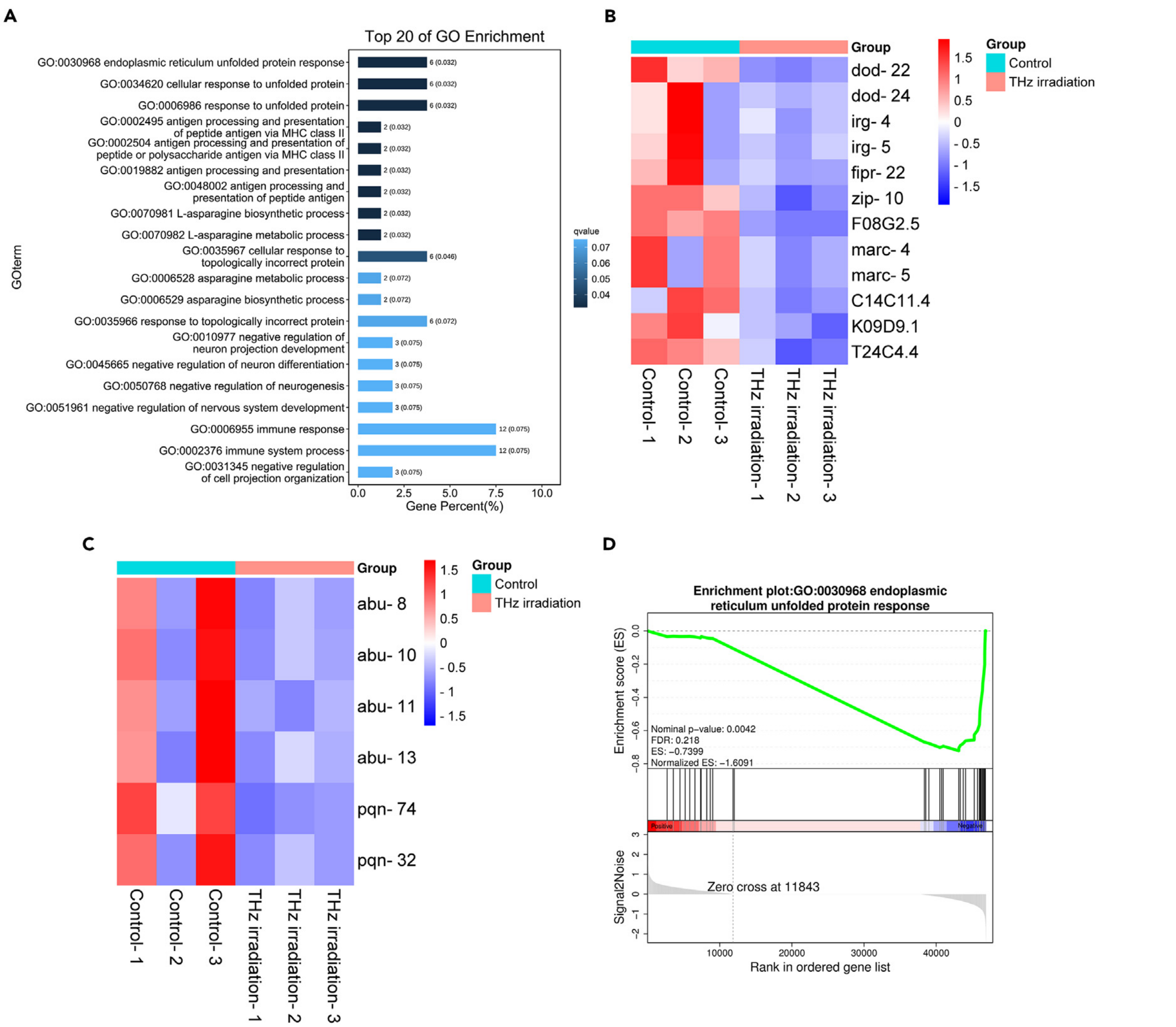

Figure 3. THz irradiation reduced the expression of genes involved in immune response

(A) The functions of the different expression genes involved in biological process by gene ontology analysis; (B) The clustering heatmap of 12 genes in immune response; (C) The clustering heatmap of 6 genes in endoplasmic reticulum unfolded protein response; (D) The enrichment plot of endoplasmic reticulum unfolded protein response gene set analyzed by GSEA analysis.

immunity of *C. elegans*. Therefore, these findings strongly implied that the innate immunity of *C. elegans* is considerably disturbed by high-power terahertz irradiation.

THz irradiation affects the genes expression involved in the nervous system

In addition to the genes involved in the immune system, the GO function analysis also showed that the genes involved in "negative regulation of neurogenesis" and "negative regulation of nervous system development" were also affected by THz irradiation (Figure 2A). THz irradiation significantly reduces the expression of slt-1 (Figure 4A) which is required for midline guidance and plays a role in the migration of neurons along the anterior-posterior axis.[19] Similarly, the gene RHGF-2, responsible for encoding a RhoGEF protein that regulates neurite outgrowth and movement in *C. elegans*,[20] was also decreased by THz irradiation (Figure 4A).

Moreover, the results of the Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis showed that the genes related to the synaptic vesicle cycle and GABAergic synapse were also changed after THz exposure (Figure 4B). Among these genes, snf-11, lgc-47, and gpc-1 were all down-regulated by THz irradiation (Figure 4C). The snf-11 gene encodes a protein that bears a strong resemblance to mammalian GABA transporters (GATs), which are involved in the clearance and/or recycling of neurotransmitters from synaptic clefts.[21] LGC-47 is an inhibitory acetylcholine receptor involved in synaptic transmission[22] and GPC-1 is a sensory G protein that modulates longevity and taste adaptation in *C. elegans*.[23] The alteration in the expression of these genes implied that neural signal transmission might be affected by THz irradiation.

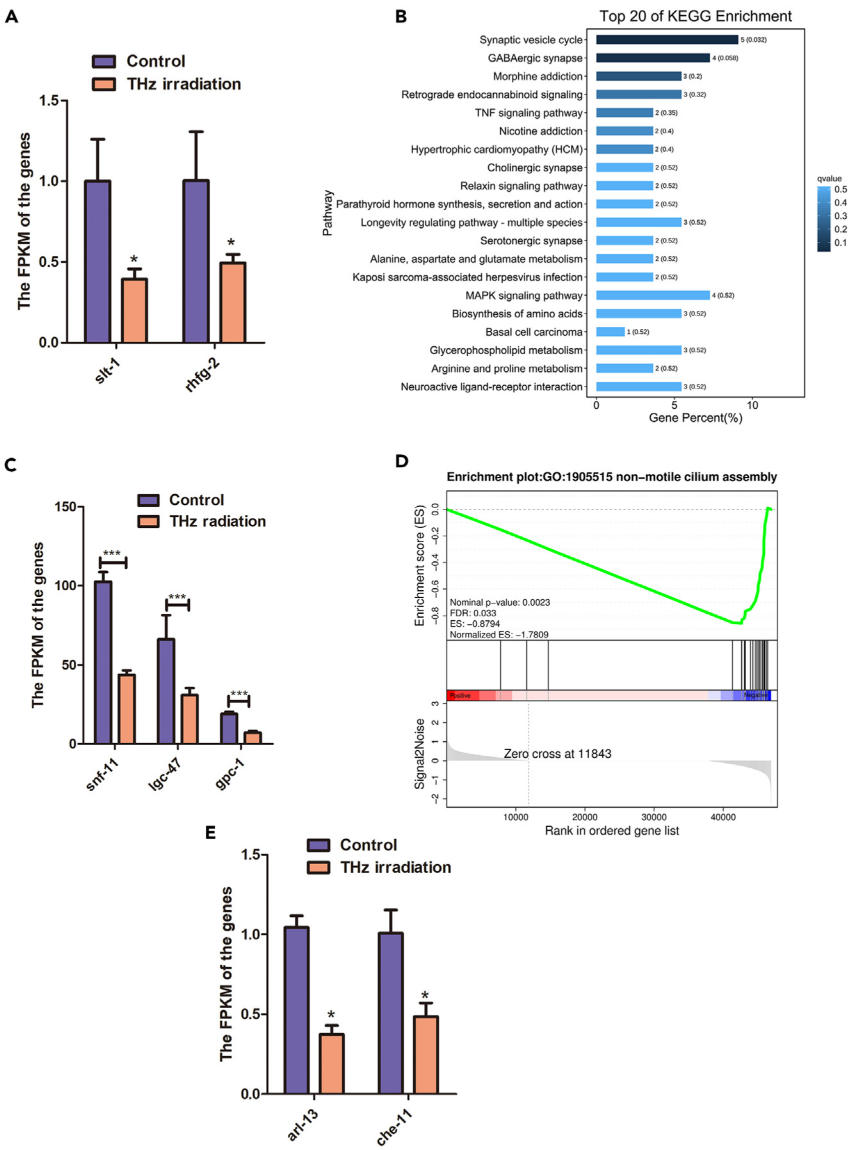

Figure 4. THz irradiation affects the genes expression involved in the nervous system

(A) The gene expression involved in nervous system development; (B) Significantly enriched KEGG pathway for differential genes between THz irradiation group and control group; (C) The gene expression involved in GABAergic synapse changed by THz irradiation; (D) The enrichment plot of non-motile cilium assembly gene set analyzed by GSEA analysis; (E) The gene expression involved in non-motile cilium assembly changed by THz irradiation; Data are shown as mean ± SEM, $^*p < 0.05$ $^{***}p < 0.001$, compared with control group.

In addition to these genes, the GSEA analysis revealed that the genes involved in "non-motile cilium assembly" showed a downward trend following THz irradiation (Figure 4D). For instance, THz irradiation significantly reduces the expression of arl-13 (Figure 4E), which encodes the protein ADP-ribosylation factor involved in cilium organization.[24] THz radiation also inhibited the expression of che-11, which is an ortholog of Chlamydomonas IFT140 and encodes a big protein with the function of positively regulating cilia intracellular transport.[25] The non-motile cilia are associated with the sensory ability of *C. elegans* to respond to chemical and physical environments.[26] Therefore, THz irradiation likely affected the function of sensory neurons due to the decreased expression of cilia genes. The alteration of all these genes involved in nervous system development and cilia assembly suggested that the neurological system of *C. elegans* was also affected by THz irradiation.

DISCUSSION

In this study, we investigated the effects of 0.26 THz irradiation with a power density of 24 mW/cm^2 on *C. elegans* by integrating transcriptome sequencing and behavioral tests to reveal the biological effects of terahertz waves at the individual level. We monitored the temperature changes at the irradiation site during the irradiation process, thereby determining that the changes in nematodes caused by terahertz were due to non-thermal effects. Our findings showed that THz irradiation resulted in damage to the cuticle structure, decreased motility and altered the expression of many genes involved in the immune and nervous systems in *C. elegans*. At the level of the transcriptome data as a whole, terahertz waves were more skewed toward lower gene expression in *C. elegans*, as down-regulation occurred in 87% of all differentially expressed genes. These genes with reduced expression are involved in a number of functions, including cuticle collagen production, the noncanonical UPR pathway, innate immune responses, and neurological activities.

The cuticle, secreted by the hypodermis, is the outermost layer of the skin,[17] and it is also the first tissue component to respond to terahertz irradiation. In this study, we found that THz irradiation reduced the expression of many collagen genes, such as col-125, col-89, and col-167, but positively regulated the expression of col-73. The cuticle biogenesis and collagen expression are regulated by several transcription factors, such as SKN-1, heat shock transcription factor (HSF-1), and TGF-β. Each of these transcription factors was reported to regulate a group of collagen genes. SKN-1 regulated collagen genes such as col-10, col-13, and col-120 et al.[27] HSF-1 promotes the expression of col-3, col-19, and col-181 etc.[28] TGF-β signaling activates the expression of col-165, col-89, col-167, etc., while it negatively regulates the expression of col-64.[29] According to our sequencing results, there was no overlap between terahertz-altered collagen genes and the genes downstream of HSF-1 and SKN-1, suggesting that the HSF-1 and SKN-1 signaling pathways were not responsible for the terahertz-induced alteration in collagen genes expression. However, the expression of two collagen genes, col-89 and col-167, which are regulated by TGF-β pathways, was reduced after THz exposure. It suggested that the TGF-β signaling was affected by 0.263 THz irradiation. It has also been reported that fs-THz radiation can regulate the expression of wound response genes by activating the TGF-β signaling pathway in mouse skin.[6] Thus, it is likely that TGF-β signaling is a key signaling pathway stimulated by diverse THz irradiation.

In this study, we observed the wrinkled cuticle of C. elegans after exposure. Actually, we also found the thickened stratum corneum in mice in another experiment when mice were exposed to 3.1 THz pulsed radiation (data not published). Our speculation is that this could be attributed to the higher peak power of pulsed radiation. Like all other forms of electromagnetic radiation, the impact of THz is intricately linked to their frequency, intensity, waveform, and the exposure duration. Different experimental conditions frequently yield varying results. Generally, THz radiation with higher power density is more likely to produce the negative or even cytotoxic effects, such as proliferative disorder, abnormal DNA damage repair, and increased apoptosis.[14,30] Given our previous studies on the bioeffects of high-power microwave pulse radiation, it was evident that even with a relatively low average power density, pulsed radiation could result in more significant effects due to the rapid change in field strength. Thus, it is still necessary to carry out more in-depth research and analysis to figure out a clearer picture of terahertz's safety.

The immune system is also sensitive to electromagnetic radiation. Recently we found that the levels of inflammatory factors, such as IL-6 and IL-1β, were significantly reduced in the serum of mice after 3.1 THz irradiation exposure (data not yet published). This suggested that THz waves impact the immune system of mice. In this study, the expression of many immune response genes, including dod-22, dod-24, irg-4, and irg-5, which encode the CUB-domain protein[18] were found to be reduced by THz irradiation in *C. elegans*. Along with the CUB-domain proteins, THz irradiation also influenced the expression of C-type lectins, such as clec-94 and clec-263 (data not shown) which were classified as surface proteins involved in recognizing carbohydrate structures of pathogens and self-antigens.[31] In addition, Ilys-3, a lysozyme that is an antibacterial protein found in microbes and insects and responsible for lysing bacterial cells by dissolving peptidoglycan,[32] was also reduced by THz irradiation. Decreased expression of these immune genes implies that the nematode's immune system is impaired after terahertz exposure, making it more susceptible to bacterial and fungal infections. The UPR is also closely tied to the normal functioning of immune defense in *C. elegans*.[33,34] In particular, the uncanonical UPR pathway is required for innate immunity. Many proteins involved in the uncanonical UPR pathway were encoded by abu genes as well as some pqn genes,[35] while the expression of several abu genes and pqn genes were reduced after THz exposure. This suggested that THz irradiation may inhibit the uncanonical UPR pathway and subsequently may lead to impairment of the innate immunity of *C. elegans*. Taken together, the altered expression of several subsets of immune-related genes as well as the impaired cuticles, all suggested that 0.263 THz irradiation has an impact on the immune system of *C. elegans*. While our findings raised some intriguing questions. Generally, damage to the body would trigger the immune response and activate the expression of immune-related genes. However, in our study, the majority of immune-related differentially expressed genes were down-regulated by THz irradiation. Therefore, further investigations are definitely needed to gain a better understanding of how THz irradiation affects the expression of immune-related genes and what the actual effects of THz are on immune functions against various challenges.

The nervous system is also sensitive to electromagnetic radiation. Several studies have demonstrated that THz waves can modulate gene expression and the activity of neurons. Our previous study showed that the differentially expressed genes in primary rat hippocampal neurons exposed to THz radiation are mainly enriched in biomolecular binding-related gene categories, such as "long-chain fatty acid binding," "GTPase binding," and "phospholipid binding". A recent study also demonstrated that primary neuron cultures exhibited a significant increase in the expression of synaptic-associated proteins, such as Homer1 and synapsin, with either short- or long-term irradiation. Meanwhile, they also found that THz irradiation promoted excitatory synaptic transmissions by increasing the average frequency of spontaneous excitatory synaptic currents (sEPSCs) in the primary neurons.[36] There is an obvious limitation in these cell-based *in vitro* experiments. For the majority of organisms, THz radiation is unlikely to have a direct interaction with neurons in a non-invasive manner. Therefore, it is hard to tell the effects of THz radiation on the nervous system *in vivo*. In this study, we revealed that several genes involved in the synaptic vesicle cycle, GABAergic synapse and the regulation of nervous system development were changed after THz irradiation in *C. elegans*. Additionally, several genes involved in cilium assembly, which contribute to nematode sensation, were also negatively regulated by THz irradiation. These results shed light on the potential impact of THz irradiation on the nervous system under whole-body exposure conditions. Naturally, the *in vivo* activities of neurons and the sensory function of *C. elegans* are expected to be further characterized to elucidate the practical effects of THz radiation on neurological functions.

In this study, we reported the changes in genes induced by THz irradiation, but the detailed mechanisms still required further study. As shown in our previous study, the binding efficiency of a transcription factor with its corresponding binding site in DNA was affected by THz irradiation.[37] This could be one of the possible approaches for THz irradiation to regulate gene expression in *C. elegans*. Additionally, the DNA breathing model could also be applied to explain and predict the effects of THz radiation on gene expression, as it suggested that THz radiation may create new open states in the DNA helix through nonlinear resonance.[38,39] What's more, the THz wave has been suggested to enhance the permeability of the voltage-gated calcium channel through a resonance effect, thus increasing Ca^{2+} permeation and ultimately resulting in the induction of gene transcription.[40] Tachizaki et al. also reported that THz pulses could affect the expression of many genes regulated by zinc-dependent transcription.[41] These findings can, to some extent, explain the possible mechanism by which THz radiation regulates gene expression. However, more research is still needed to fully understand the precise mechanisms.

Overall, our findings in this study, obtained by integrating transcriptome sequencing and behavioral tests of *C. elegans* revealed the effect of high-power THz irradiation on intact *C. elegans*. In addition to the clearly damaged appearance of the epidermal ultra-structures and the altered cuticle collagen genes, reduced gene expressions related to immune and neural functions were the main effects of THz irradiation in *C. elegans*. This hints at the possibility that the immune and neurological systems might exhibit greater sensitivity to THz irradiation when subjected to whole-body exposure. Moreover, our study also offered new evidence indicating the potential health risks associated with specific THz radiation. Therefore, it is still necessary to carry out more in-depth research and analysis to figure out a clearer picture of terahertz's safety. Hence, further comprehensive research and analysis are required to gain a better understanding of the bioeffects and biosafety of THz radiation.

Limitations of the study

As mentioned in the discussion, in this study, we reported the changes in genes induced by THz irradiation, but the potential biophysical mechanism of 0.263 THz irradiation on the gene expression change of *C.elegans* needs further investigation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

Conceptualization, X.L.and S.S.; methodology, S.S. and F.G.; software,T.S.; validation, W.W. and F.G.; investigation, S.S., W.W., and F.G; resources, S.T.; writing-original draft preparation, S.S.; writing-review and editing, S.S.; supervision, X.L., Y.G., and D.L.: project administration, L.Y.; funding acquisition, X.L. All authors have read and agreed to the published version of the manuscript."

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Agar	sigma	9002-18-0
Peptone	sigma	73049-73-7
Sodium chloride	sigma	7647-14-5
Cholesterol	sigma	57-88-5
Trizol	Invitrogen	15596026
Calcium chloride	sigma	10043-52-4
Magnesium Sulfate	sigma	7487-88-9
KH2PO4	sigma	7778-77-0
M9 buffer	GENMED SCIENTIFICS INC	GMS12302.1
glutaraldehyde	sigma	1.04239
Ethanol	sigma	E7148
tert-butyl alcohol	sigma	BX1805
Experimental models: Organisms/strains		
Wild-type *C. elegans (Bristol strain, N2)*	Kindly provided by Zhiyong Shao	
Escherichia coli OP50	Kindly provided by Zhiyong Shao	
Software and algorithms		
GraphPad Prism 7.0	GraphPad Software	https://www.graphpad.com/
R for Windows 4.3.2	R software	https://www.r-project.org/
Other		
Infrared thermometer	FOTRIC	FOTRIC 323PRO

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xiaoyun Lu (luxy05@xjtu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: Data reported in this paper will be shared by the lead contact upon request. The raw data and clean data of the RNAseq in this experiment were all uploaded in the GEO Database: GSE243842.
- Code: This paper does not report original codes.
- All other items: Any additional information required to reanalyzed the data in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The experimental model used in this experiment was the wild-type *C. elegans* named Bristol strain, N2 which was kindly provided by Professor Shao Zhiyong (Fudan University, China). The food of *C. elegans Escherichia coli* OP50 used in this experiment was also provided by Professor Shao Zhiyong.

METHOD DETAILS

Nematode strains and culturing

Wild-type *C. elegans* (Bristol strain, N2) was kindly provided by Professor Shao Zhiyong (Fudan University, China), and grown at 20°C on nematode growth medium (NGM) agar plates (1.7% agar, 2.5 g/L peptone, 25 mmol/L NaCl, 25 mmol/L KPO4 buffer (pH 6.0), 5 mg/L cholesterol, 1 mmol/L $CaCl_2$) seeded with *Escherichia coli* OP50.

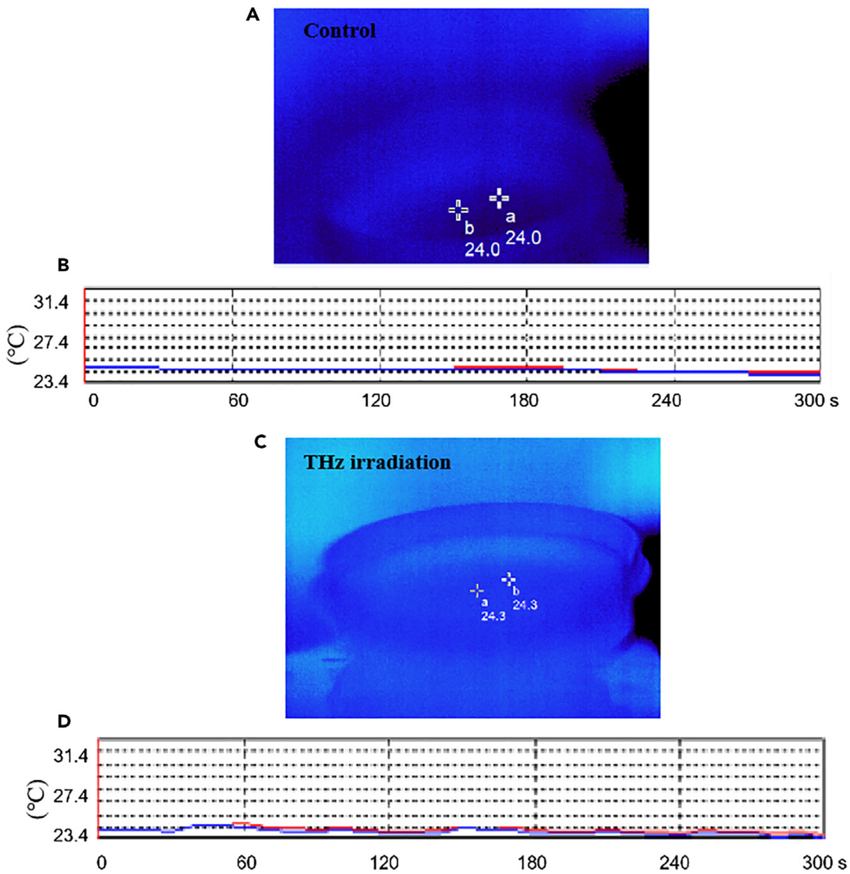
Synchronization of nematodes

Synchronized populations of nematodes were initiated by conducting timed lays, during which 15–20 gravid adults were allowed to lay eggs on NGM plates for 2.5 h. Afterward, the adults were removed and the resulting eggs were allowed to hatch and develop at 20°C. To ensure transcriptome sequencing, each group of worms was cleaned from NGM agar plates and deposited in a 1.5 mL centrifuge tube, with the worm bodies covering the bottom of the centrifuge tube and approximately one hundred of nematodes. On the third day after hatching (day 3), the worms were irradiated with the THz source.

THz sources and irradiation

The exposure of *C. elegans* to terahertz radiation was carried out using the terahertz device developed by the Terahertz Science and Technology Research Center, at the School of Electronic Science and Engineering, University of Electronic Science and Technology of China. The beam current of the THz source is 400 mA, the beam voltage is 16.66 kV, and the operating magnetic field is 9.6 T. The gyrotron operates in TE72 mode and is converted to Gaussian mode by a quasi-optical mode converter. The Gaussian mode is transmitted to *C. elegans* by a THz transmission line. The gyrotron frequency is 0.263 THz, with a pulse of 100 μs and a repetition frequency of 50 Hz. The average power of THz wave on the *C. elegans* is 300 mW. The synchronized young adult worms were cultivated on a 6.5 cm plate and exposed to THz irradiation for 5 min. The exposure set-up is schematically depicted in below figure.

The schematic diagram of the irradiation device



Variation of temperature with irradiation time

Two points, "a" and "b" in the plate center are selected to characterize the temperature change tracked by the infrared thermometer. (A) the control plate; (C) the THz irradiation plate; (B) is the temperature changes in the control plate (A); (D) is the temperature changes in the THz irradiation plate (C). The red line indicated the temperature of "a" point, while the blue line indicated the temperature of "b" point in (B) and (D).

An infrared thermometer was used to track the temperature changes in the irradiation area. The second figure below shows the temperature variation with irradiation duration. The findings indicated that there was no discernible change in temperature in the irradiated area during the 5-min irradiation period.

Transcriptome sequencing and data analysis

The 3 irradiated and 3 control samples were washed with M9 buffer 5–6 times to remove bacteria and the worms were then used to extract the total RNA using Trizol (Invitrogen, Waltham, MA, USA). The quantity of total RNA was detected by an Agilent 2100 Bioanalyzer (Agilent Technologies, USA), and the quality and integrity were assessed by a NanoDrop spectrophotometer. RNA was then used to prepare the library for transcriptome sequencing. The raw data was cleaned of adaptors and low-quality sequences using TrimGalore to obtain clean data. The sequence reads were aligned to the reference genome (Ensembl WBcel235) using Soap 2.21 software. Normalized gene expression was performed by calculating the number of RNA-Seq fragments per kilobase of transcript per total million fragments mapped. Cuffdiff and CummeRbund were used to identify the differentially expressed genes (DEGs) and to plot expression, respectively. The significantly expressed genes were selected based on a fold change of ≥ 1.50 or ≤ 0.67 and a p value of <0.05. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to analyze the significant pathways involved in THz radiation. Gene Set Enrichment Analysis (GSEA) with the C5 (Ontology gene sets) was used to analyze the GO gene sets. The RNA-seq data in this paper is available under the NCBI GEO: GSE243842.

Morphological damage assay following THz irradiation

After THz irradiation, the worms were harvested by centrifugation and washed with M9 buffer 3–4 times. The epidermal ultrastructures were observed with a scanning electron microscope (SEM). The worms were fixed overnight with 2.5% glutaraldehyde (EM grade) and dehydrated in a series of ethanol solutions and *tert*-butyl alcohol. Then the worms were critical-point dried with CO_2 and coated with gold before being examined under the SEM at 20 kV.

Locomotion assay following THz irradiation

The endpoints of body bends reflect locomotion behaviors of *C. elegans*.[42] To assess body bends, the nematodes were picked up onto a new plate with M9 buffer. After a 1-min recovery period, body bends were counted for 20 s. A body bend was calculated as a change in the direction of the part of *C. elegans* corresponding to the posterior bulb of the pharynx along the y axis, assuming that *C. elegans* moves along the x axis. Thirty nematodes were examined per replicate.

QUANTIFICATION AND STATISTICAL ANALYSIS

The data were processed in Excel and the statistical significance of differences was analyzed using of Prism 5 software (GraphPad Software, USA). Student's *t* test was used to compare two groups. The data are presented as means $\pm$ S.E.M, and a p value of < 0.05 was considered statistically significant.