Dosage-dependent Induction of Behavioral Decline in *Caenorhabditis elegans* by Long-term Treatment of Static Magnetic Fields

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Nematode/Apoptosis/Static magnetic field/Gene expression.

The aim of this work was to explore the molecular mechanisms associated with possible health hazards induced by static magnetic fields (SMFs). Nematodes were grown under SMFs at field strengths from 0 to 200 mT, and the speed of body movement was measured. The effects of exposure to static magnetic fields were observed to be significant in the higher field strength and longer treatment. To explore the possible molecular mechanisms responsible for these effects, semi-quantitative real-time RT-PCR was performed using primers specific to 120 randomly selected genes. Twenty-six differentially expressed genes among apoptosis-, oxidative stress-, and cancer-related genes were identified, indicating that a global molecular response to SMF treatment occurred. The induction of apoptosis was verified by the increase of fluorescence in a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, by the caspase-3 activity assay, and by immunostaining using an antibody against the ced-3 gene product. Mutations in genes involved in major apoptotic pathways, that is, ced-3, ced-4, and ced-9, abolished this SMF-induced behavioral decline; this is consistent with the hypothesis that the apoptosis pathways are involved in the SMF-induced mobility decline. Here we show that long-term and low-dosage exposure to SMF is capable of inducing an apoptosis-mediated behavioral decline in nematodes.

INTRODUCTION

Human beings use electricity freely for convenience and the improvement of life. Possible undesirable effects of electric and magnetic field exposure have raised great concerns. Most of the epidemiological research has focused on exposure to power frequency magnetic fields or radiofrequency fields; however, there have been only a few epidemiological studies specifically designed to study the potential health hazards of static magnetic field exposure. It is of general concern that the present knowledge regarding the health concerns of static magnetic fields (SMFs) is lagging behind medical development such as magnetic resonance imaging (MRI). New studies are needed to fill the gaps in knowledge and provide assurance that novel medical technology will not cause any unwanted health hazards.

There have been studies on the effects of SMFs at the cellular level. Static magnetic fields alone do not have lethal effects on the basic properties of cell growth and survival under normal culture conditions. Most studies also suggest that SMFs fail to affect the proliferation of cells or to influence the cell cycle. However, disturbances in gene expression have been reported. In HeLaS3 cells, SMFs enhanced c-Fos expression as examined by Northern blot. Morphological analyses indicate that SMFs induce modifications in cell shape, cell surface, and cytoskeleton. Enhanced expression of eNOS and VEGF in endothelial cells is reported. SMFs may also modulated apoptosis via an influence on the cytoplasmic calcium ion concentration.

In multicellular organisms, the minor effects of SMFs at the cellular level may accumulate and result in distinct symptoms. Animal studies indicate SMF induces flow potentials around the heart; however, effects on reproduction and development are not clear. Since *Caenorhabditis elegans* provides is a useful and valuable model animal in the genetic and molecular investigation of multicellular organisms, this nematode may provide insights that will help fill the gaps in knowledge regarding possible health hazards. For *C. elegans*, short-term treatment with SMFs induced fluctuations in heat shock protein gene expression, but in general, low genotoxicity was observed.

We have previously shown that when nematodes are
 grown under 200 mT SMF, the development time is shortened by approximately 25% from L2 larva to the young adult stage. The lifespan was reduced from 31 days to 25 days.24) Apparently, SMF is capable of accelerating the development and shortening the lifespan of nematodes.25) It is of particular interest to investigate molecular mechanisms causing the acceleration of development and the reduction in lifespan. Since the decline in body movement is associated with the aging process, we intended to follow this phenotype and to dissect pathways that may elucidate the SMF-induced health hazards. The current study was based on the hypothesis that SMF may induce an abnormality in nematode and that this abnormality may be correlated to molecular pathways such as apoptosis.

**MATERIALS AND METHODS**

**Strains and chemicals**

The strains of *C. elegans* used in this research, including wild-type (N2), *ced-3* (n717), *ced-4* (n1162), *ced-6* (n1813), *ced-9* (n1950), and *cbp-1* (ku258), were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota, St Paul, Minnesota, USA. Populations of *C. elegans* exist primarily as hermaphrodites. The mutant strain *ced-9* (n1950) carries gain-of-function mutation, 16 which prevents somatic cell deaths in *C. elegans* by blocking the translocation of CED-4 in 17 mitochondria. Nematodes were propagated at 16°C on nematode growth medium (NGM) plates26) with Escherichia coli strain OP50 as a food source. All culture media and related chemicals including Bacto agar, Bacto tryptone, and yeast extract were purchased from Gibco Co. Other chemicals of analytical grade or higher were purchased from Sigma or Merck.

**Static magnetic field device for culturing nematodes**

The SMF device was constructed as described previously.24) The SMF device was composed of two Nd-Fe-B permanent magnets that sandwich a Petri dish of 6 cm in diameter and 1 cm thick (Fig. 1). The dimensions of magnet were 10 cm × 10 cm × 1 cm. Nd-Fe-B magnets were purchased from Taiwan Magnetic Corp. Ltd. The magnetic field strength at the center of the SMF device was measured at the National Measurement Laboratory, Taiwan. The distance between the magnets and plate was adjusted to obtain field intensities from 0 to 200 mT at the center of plate. During experiment, nematodes were seeded on the top of agar. SMF device was assembled with proper distance adjustment. The complete device was placed in the incubator and the temperature was set at 16°C.

**Mobility assay**

To effectively capture the locomotion behavior of a freely moving worm, a grid paper was placed underneath the Petri dish, and the movement of worms was recorded using an optical microscope equipped with a CCD camera. The moving speed was obtained by analyzing the recorded video. Number of sine waves per minute (sw/min) propagated along the anterior/posterior axis in the dorsal/ventral plane was also analyzed. The measurement was performed on 30 worms, and the result was expressed as mean ± SD (standard deviation). An adult nematode travels at a speed of approximately 8 mm/min with mobility of 12 sw/min.

**Semi-quantitative real-time RT-PCR**

Total RNA was extracted from 100 worms using TRI-reagent (Talron Biotech) according to the manufacturer’s specifications. Worms werepicked and washed three times with M9 media and collected in a 2 ml Eppendorf tube. The pellet was dissolved in 1 ml TRI-reagent and disrupted using a homogenizer on ice, followed by chloroform extraction and isopropanol precipitation. The crude RNA extract was immediately purified using an RNeasy Mini Kit (Qiagen) to remove impurities and unwanted organic compounds. Purified RNA was resuspended in DEPC water and quantified by OD260. The OD260 to OD280 ratio usually exceeded 2.0 at this stage. For cDNA synthesis, 1 μg total RNA was annealed with 1 μg oligo-dT, followed by reverse transcription using SuperScript® III Reverse Transcriptase (Invitrogen) in a total volume of 50 μl. Between 0.2 and 0.5 μl of the reverse transcription reactions were used for semi-quantitative real-time PCR using SYBR Green 1 on an iCycler iQ5 (Bio-Rad Laboratories). Cycling conditions were as follows: 1x [5 min at 95°C] and 50x [20 s at 95°C, 20 s at 60°C, 40 s at 72°C]. The fluorescence was measured after each 72°C step. Expression levels were obtained as threshold cycles (Ct) determined by iCycler iQ Detection System software. The PCR efficiency among the tested genes and control genes were the same for most genes, performed based on the protocol provided by Bio-Rad. If the target and the reference genes do not have similar amplification efficiencies, Relative Expression Software Tool (REST)27) was used for correction. The PCR primers are designed based on the software and sequence deposited in NCBI database.

Relative transcript quantities were calculated by the ΔΔCt method using ribosomal proteins L18 and L21 as reference genes amplified from the same cDNA sample. ΔCt is the difference in threshold cycles of the sample mRNAs relative to ribosomal protein L18 or L21 mRNA. ΔΔCt is the difference between the ΔCt of the normal control and the ΔCt of the
SMF-treated sample. The fold change in mRNA expression was expressed as \(2^{\Delta\Delta Ct}\). Fold data was used for statistical analysis. Results are expressed as mean ± SD of six experiments.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay
A TUNEL assay was performed using the APO-BrdU™ TUNEL Assay Kit (Invitrogen) according the manufacturer’s specifications with minor modifications. Worms were washed three times with M9 media and collected in a 2 ml eppendorf tube. Worms were fixed in 4% formaldehyde for 15 minutes at room temperature, followed by washing with PBS three times. Worms were further incubated in 100 μl of a 20 μg/ml Proteinase K solution for 10 minutes at RT, washed in PBS for 5 minutes, fixed in 4% formaldehyde for 5 minutes at RT, washed in PBS for 5 minutes, and incubated in 100 μl Equilibrate buffer for 10 minutes at RT. The fixed worms were resuspended in 100 μl TdT reaction mix (90 μl equilibrate buffer, 10 μl nucleotide mix, and 2μlTdT enzyme) and incubated at 37°C for 1 hour in a humidified dark chamber, followed by one wash with 2 × SSC and three washes with PBS. The fluorescence imaging was performed with a Leica TCS SP scanning confocal microscope.

Immunostaining
Immunostaining was performed based on the procedure described previously with minor modifications. Worms were washed three times with M9 media and then fixed with 4% formaldehyde for 10 minutes at RT. The cell membrane was disrupted by adding 0.1% Triton X-100 diluted in 0.1% BSA for 30 minutes at RT. After washing with PBS, the worms were incubated with a mouse anti-CED-3 IgG diluted 1:200 in 0.1% BSA overnight at 4°C. The worms were washed three times with PBS and were incubated with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG diluted 1:1000 in 0.1% BSA for 1 hour at RT, followed by washing with PBS three times. The fluorescence imaging was performed with a Leica TCS SP scanning confocal microscope, with the excitation wavelength set to 496 nm and emission set to 521 nm.

Caspase-3 activity assay
Caspase-3 activity was evaluated using the CaspACE assay system according to the protocol provided by the Promega. The nematodes plated on the agar plate were treated with M9 buffer, were then harvested, washed, and homogenized for 15 min. The samples were then centrifuged at 15000 xg for 20 min at 4°C. The cell lysate supernatant was aliquoted into the wells of a 96-well plate, and 32 μL buffer, 2 μL DMSO, 10 μL DTT (100 mmol/L), and 2 μL of DEVD-pNA (10 mmol/L) were added to a final volume of 100 μL. The samples were incubated for 4 h at 37°C. The absorbance at 405 nm was measured and enzyme activity was calculated as pmol pNA liberated per hour/μg protein according to the formula provided by the manufacturer.

Statistical analysis
All data were presented as means ± standard error of the mean (SEM). Group means for crawling speed were evaluated using one-way analysis of variance (ANOVA). If the data were homogenous and normally distributed, multiple comparisons were made by Newman-Keuls test. If tests for normality or variance failed, the Kruskal-Wallis one-way ANOVA on ranks was carried out. The minimum level of significance was set at P < 0.05 or at P < 0.01. Student’s t-test was performed when two groups were compared in the mutant analysis.

RESULTS

SMF induces behavioral decline in nematodes
The decline of physiological processes in Caenorhabditis elegans is closely associated with the normal aging process in humans. To explore possible the health hazards of static magnetic fields (SMFs), nematodes were grown under SMFs at field strengths from 0 to 200 mT, and the speed of body movement was measured. A time course experiment was then performed using synchronized wild-type N2 nematodes under SMFs ranging from 0 to 200 mT (Fig. 2). At room temperature, adult N2 nematodes move approximately at 8 mm/min with a mobility of 12 sw/min. Extended SMF...
Static Magnetic Fields Reduce Nematode Mobility

Treatment affected the crawling action of the worms. The reduction in moving speed and mobility was dosage-dependent and time-dependent. SMFs under 50 mT had no significant effect. A significant reduction in mobility occurred when the field strength was higher than 150 mT for treatment longer than 4 days. The moving speed reduced to 1.5 mm/min under our most extreme condition (200 mT for 8 days, Fig. 3).

**Apoptotic genes were differentially expressed in the presence of SMF**

The reduced moving speed of nematodes indicated a possible decline of the physiological function related to movement, such as a problem with muscle cells or neuronal cells. It is likely that the gene expression of those cells was altered by SMF treatment. To screen for genes that are differentially expressed under SMF treatment, semi-quantitative real-time RT-PCR was performed. For the preliminary screen, 120 genes were randomly selected from among genes related to cancer, apoptosis, development, stress responses, and metabolism. Among these genes, 26 consistently showed differential expression (Table 1). These genes are associated with apoptosis (ced-2, ced-3, ced-6, ced-8), cancers (abl-1, cbp-1), stress responses (hsp16, hsp70, hsp90), and aging (age-1).

Of greatest interest, is the enhanced effect on genes associated with apoptosis. Apparently, the apoptotic pathways play a role in the SMF-induced mobility reduction of nematodes.

Biochemical evidence revealed an increase of apoptotic activity under the influence of an SMF

The abnormality of apoptosis was further verified by functional assays. One of the characteristics of apoptosis is the degradation of DNA after the activation of Ca/Mg-dependent endonucleases. This DNA cleavage leads to strand breaks within the DNA. The TUNEL method detects the broken ends of DNA in situ using terminal deoxynucleotidyl transferase (TdT). Fluorescent TUNEL staining of the SMF-treated *C. elegans* was performed (Fig. 4A–D). Cytochalasin D was administered as a positive control for apoptosis. Cytochalasin D inhibits actin polymerization and induces depolymerization of the actin filaments formed during platelet shape change. Cytochalasin D treatment triggers premature apoptosis of insect ovarian follicle and nurse cells. Cytochalasin D-treated nematodes showed global TUNEL staining over the whole body. SMF treatment, on the other hand, induced localized apoptotic events. Fluorescence was distributed along both sides of intestinal adducts of the nematode.

![Fig. 3. Crawling speed and mobility of nematodes treated by SMFs. The upper panel shows the effects of 4 days SMF treatment on crawling speed (left) and mobility (right). The lower panel shows the effects of 8 days SMF treatment on crawling speed (left) and mobility (right). The results are expressed as the mean ± SD calculated from 30 worms. Multiple comparisons were carried out by Newman-Keuls test if the data were normally distributed or by Kruskal-Wallis one-way ANOVA if tests for normality or variance failed. The minimum level of significance was set at P < 0.05 (*) or P < 0.01 (**).](image-url)
Immunostaining using an antibody against the gene product of ced-3 was also performed (Fig. 5). Ced-3 encodes a gene product that plays essential role in apoptosis. Positive staining for the SMF-treated nematodes confirmed the occurrence of apoptosis. The results of both the TUNEL staining and immunostaining assays are consistent with the hypothesis that SMF induced apoptosis in nematodes thus affected the mobility of the worms.

Caspase 3 activity is characteristic of cells undergoing apoptosis. Caspase 3 is a caspase protein that interacts with caspase 8 and caspase 9. Caspase 3 is encoded by the CASP3 gene. CASP3 orthologs have been identified in numerous mammals for which complete genome data are available. Unique orthologs are also present in birds, lizards, lissamphibians, and teleosts. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small.

### Table 1. List of differentially expressed genes as determined by semi-quantitative RT-PCR

| Symbol | Annotation                                         | ΔΔCt | SD | Fold |
|--------|----------------------------------------------------|------|----|------|
| Abl-1  | Related to oncogene abl                            | 5.56 | 0.17 | 47.18 |
| Alix-1 | Alix (apoptosis-linked gene 2 interacting protein x) homolog | –1.32 | 0.26 | 0.40 |
| Bir-1  | Bir (baculovirus inhibitory repeat) family         | 2.43 | 0.51 | 5.39 |
| Ced-3  | Cell death abnormality                             | 4.16 | 1.00 | 17.88 |
| Ced-2  | Cell death abnormality                             | 2.71 | 0.36 | 6.54 |
| Ced-6  | Cell death abnormality                             | –0.71 | 0.22 | 0.61 |
| Ced-8  | Cell death abnormality                             | 4.27 | 1.64 | 19.29 |
| Che-13 | Abnormal chemotaxis                                | –0.641 | 0.09 | 0.64 |
| Mel-26 | Maternal effect lethal                             | 3.43 | 0.88 | 10.78 |
| T27F7.2 | Shc-2 - (shc/src homology domain c-terminal adaptor homolog) | 2.05 | 0.68 | 4.14 |
| Tir-1  | Tir (toll and interleukin 1 receptor) domain protein | 4.83 | 0.50 | 28.44 |
| Nft-1  | Nithit family                                      | 1.06 | 0.56 | 2.08 |
| Par-4  | Abnormal embryonic partitioning of cytoplasm       | 4.63 | 0.42 | 24.76 |
| Bub-1  | Yeast bub homolog                                  | 5.98 | 0.34 | 63.12 |
| Daf-18 | Abnormal dauer formation                           | 2.35 | 0.46 | 5.10 |
| Cbp-1  | Cbp/p300 homolog                                   | 3.23 | 0.88 | 9.38 |
| Dic-1  | Human dice1 (deleted in cancer) homolog            | 5.5 | 0.43 | 45.25 |
| Hoe-1  | Homolog of elac2 (cancer susceptibility locus)      | –1.35 | 0.29 | 0.39 |
| Cyp-4A1 | Cytochrome p450 family                            | –1.82 | 0.53 | 0.28 |
| Sod-2  | Sod (superoxide dismutase)                         | 2.14 | 1.22 | 4.41 |
| Hsp 16 | Heat shock protein                                 | 2.67 | 0.78 | 6.36 |
| Hsp 70 | Heat shock protein                                 | 2.94 | 0.22 | 7.67 |
| Hsp 90 | Heat shock protein                                 | 3.68 | 0.57 | 12.82 |
| Act-1  | Actin                                              | 4.85 | 0.65 | 28.84 |
| Age-1  | Ageing alteration                                  | 1.86 | 0.98 | 3.63 |
| Dif-2  | Differentiation abnormal                           | 5.62 | 1.24 | 49.18 |

aThis list contains 26 genes that were selected from the real-time PCR results of 120 genes. The selected genes were consistently differentially expressed, with p-values less than 0.05 in the Student’s t-test. Fold change is used in the statistical analysis.

bΔΔCt: Relative transcript quantities were calculated by the ΔΔCt method using ribosomal protein L18 and L21 as reference genes amplified from the same sample. ΔCt is the difference in threshold cycles of the sample mRNAs relative to ribosomal protein L18 or L21 mRNA. ΔΔCt is the difference between the ΔCt values of the normal control and of the treated sample. The values are averaged from 6 sets of independent experiments.

cSD: Standard deviations were calculated from results of 6 experiments.

dFold: Fold change in gene expression was calculated by the 2^ΔΔCt method.
Fig. 4. Fluorescence TUNEL assay analysis of C. elegans under the influence of SMFs. (A) Worms without SMF, cytochalasin D treatment but with the whole process of TUNEL assay as a negative control. (B) Worms were treated with cytochalasin D (0.5 μg/ml) to induce apoptosis, and these worms served as a positive control. (C) Worms were treated by 200 mT SMF for 4 days. (D) Worms same as (C) but at a higher magnification to show the localization of the fluorescence.

Fig. 5. Immunostaining of SMF-treated nematodes. Immunostaining using an antibody against Ced-3 was performed. (A) Worms without SMF, cytochalasin D treatment but with the whole process of immunoassay as a negative control. (B) Worms were treated with cytochalasin D (0.5 μg/ml) to induce apoptosis and served as a positive control. (C) Worms were treated with 200 mT SMF for 4 days. (D) The quantitative results of the caspase-3 activity.
small, that dimerize to form the active enzyme. This protein cleaves and activates caspases 6, 7, and 9, and the protein itself is processed by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of the amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer’s disease. Alternative splicing of this gene results in two transcript variants that encode the same protein.\(^{10}\) The quantification of caspase 3 activity was performed for SMF-treated nematodes using cytochalasin D as positive control for apoptosis. SMF treatment induced a significant apoptotic reaction in nematodes in comparison with the no SMF treatment group (Fig. 5D).

**The major apoptotic pathways mediate SMF-induced mobility reduction**

Although apoptosis was involved in the mobility reduction of SMF-treated nematodes, it is yet to be demonstrated if apoptosis plays a central role of this mobility reduction. We took the advantage of the mutant nematodes that carry mutations at genes involved in the apoptotic pathways. Mutant strains of ced-3 (n717), ced-4 (n1162), ced-6 (n1813), ced-9 (n1950), and cbp-1 (ku258) were tested for their resistance to SMF. The ced-3, ced-4, and ced-9 genes encode gene products that belong to the major apoptotic pathways. Ced-6 does not participate in apoptosis directly; however, ced-6 is involved in pathways that regulate cell corpse engulfment. Cbp-1 encodes a homolog of the mammalian transcriptional cofactors CBP and p300, which have been shown to possess histone acetyltransferase activity, and which, when mutated, lead to Rubinstein-Taybi syndrome and colorectal cancer.\(^{35}\)

The mobility of these mutant nematodes was measured after treatment with 200 mT SMF for 4 days (Fig. 6). For wild-type nematodes, SMF treatment induced a 41% reduction in the moving speed, from 8 mm/min to 5 mm/min, a significant reduction. Nematodes with mutations in genes that are directly involved in the main apoptotic pathways, that is, ced-3, ced-4, and ced-9, were insensitive to SMF treatment. On the other hand, the mobilities of ced-6 and cbp-1 mutants were significantly reduced, by 40% and 45%, respectively, by SMF treatment. Apparently, the major apoptosis pathways mediated the SMF-induced mobility reduction of nematodes (Fig. 6).

We have demonstrated that apoptosis may be directly involved in the behavioral decline of SMF-treated nematodes. Immunostaining using an antibody against ced-3 and fluorescence TUNEL staining indicated that apoptosis occurred at the muscle cells of the worms. However, it remains to be proven that besides mobility SMF may be capable of inducing other abnormalities such as the incidence of cancer.

**DISCUSSION**

The impacts of static magnetic fields (SMFs) on biological systems have been investigated for decades; however, the results are controversial.\(^{36}\) Drinker and Thompson have investigated the possible health hazard of SMFs on nerve muscle preparations and on living animals. They concluded that the magnetic field has no significance as a health hazard.\(^{37}\) Practically, it is difficult to prove no side effects of SMF for humans.\(^{38}\) In 2003, the FDA declared that MRI clinical systems generating static fields up to 8 T have a “nonsignificant risk status”.\(^{39}\) Nevertheless, an increase in blood viscosity due to exposure to a 1.5 T magnetic field has been observed.\(^{40}\) At the cellular level, growth, proliferation, cell cycle distribution and apoptosis seem not to be affected by exposure up to four days at 10 T;\(^{41}\) while an exposure of 10–17 T for 30–60 minutes can reduce cell number, size, organization and vitality.\(^{41}\) The available data do not allow one to reach a conclusion about the health effects of the SMF.\(^{38}\)

SMF potentially enhances cellular oxidative stress and may consequently reduce the function of skeletal muscles. Skeletal muscle differentiation was enhanced by improving myoblast alignment using a 80 mT SMF.\(^{42}\) The exposure of rats to a 128 mT SMF decreased the activities of glutathione peroxidase and superoxide dismutase in heart muscle. Subchronic exposure to SMF has increased the malondialdehyde (MDA) concentration in rat cardiac muscle.\(^{43}\) The exposure of rats to a 128 mT SMF induced an increase in norepinephrine content in gastrocnemius muscle but had no effect at 67 mT, and there was non-significant increase of HSP72.

![Fig. 6. Effect of an SMF on the mobility of wild-type and mutant nematodes.](image-url)

The black bars represent the mobility of worms without SMF treatment. The shaded bars represent the crawling speed of worms with SMF treatment. The result is the mean ± SD from 30 worms. Student’s t-test was performed to compare the crawling speeds of SMF-treated group to the untreated group. (*) represents p < 0.05.
levels in gastrocnemius muscles. The noradrenergic system in the rat gastrocnemius muscles may be affected by SMF exposure.\textsuperscript{45,46} In vertebrates, animal behavior is affected by exposure to SMFs.\textsuperscript{35,40} We have shown differential expression of Cyp-44A1, Sod-2, Hsp16, Hsp70, Hsp90, and Act-1, consistent with the increase of oxidative stress in muscle cells. Exposure of rats to 7T SMF causes locomotor circling and the suppression of rearing,\textsuperscript{47} which is consistent to the current study.

Genetic analysis using mutant strains indicated that the apoptotic pathways mediate the SMF-induced mobility reduction. Treatment with SMF up-regulates EGL-1 activity, which suppresses the expression of CED-9 (Fig. 7). The downstream proteins CED-4 and CED-3 are negatively controlled by CED-9. The up-regulation of CED-3 and CED-4 induces apoptosis in \textit{C. elegans}. The decrease in mobility may be attributed to cell apoptosis. We have shown enhanced expression of CED-3 and related genes by real-time RT-PCR. The mobility of ced-9 (n1950), ced-3 (n717), and ced-4 (n1162) mutant nematodes was not affected by SMF treatment, indicating that these genes are directly involved in the scheme. Expression of ced-4 and ced-9 did not show significant difference in our primary semi-quantitative RT-PCR screening, thus were absent in Table 1. Nevertheless, mobility assay of mutant strains strongly suggests these two genes are involved in the SMF-induced behavioral decline. CED-6, however, was affected by SMF treatment, indicating that CED-6 is not involved in the SMF-induced mobility reduction pathway. Magnetic fields, when given sufficient intensity and duration, induce apoptosis and cause damage to nematodes.

We have previously reported that SMF accelerates developmental process and reduces the lifespan of nematodes.\textsuperscript{24} Here, we show that the application of an SMF to nematodes at the strength above 150 mT for longer than 4 days significantly reduced the moving speed of \textit{C. elegans}. Using real-time RT-PCR, we were able to identify 26 genes whose expression was affected by SMF-treatment. These genes are associated with apoptosis, cancer, the stress response, and aging. A TUNEL assay and immunostaining for caspase 3 validated the occurrence of apoptosis in the worm body. Apparently, long-term and low-dosage treatment of SMF can cause declining hazards in nematodes. Understanding the general hazardous effects of SMFs on living organisms requires more intensive investigation of the underlying molecular mechanisms.

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**REFERENCES**

1. Calle EE and Savitz DA (1985) Leukemia in occupational groups with presumed exposure to electrical and magnetic fields. N Engl J Med 313: 1476–1477.
2. Englund A, Ekman G and Zabrielski L (1982) Occupational categories among brain tumor cases recorded in the cancer registry in Sweden. Ann N Y Acad Sci 381: 188–196.
3. Feychting M, Forssen U and Floderus B (1997) Occupational and residential magnetic field exposure and leukemia and central nervous system tumors. Epidemiology 8: 384–389.
4. Wertheimer N and Leeper E (1979) Electrical wiring configurations and childhood cancer. Am J Epidemiol 109: 273–284.
5. Leszczynski D (2005) Rapporteur report: cellular, animal and epidemiological studies of the effects of static magnetic fields relevant to human health. Prog Biophys Mol Biol 87: 247–253.
6. Miyakoshi J (2005) Effects of static magnetic fields at the cellular level. Prog Biophys Mol Biol 87: 213–223.
7. Nakahara T, \textit{et al} (2002) Effects of exposure of CHO-K1 cells to a 10-T static magnetic field. Radiology 224: 817–822.
8. Sakurai T, Terashima S and Miyakoshi J (2009) Effects of static magnetic fields on cell cultures. Micron 40: 1–8.
9. Hiraoka M, \textit{et al} (1992) Induction of c-fos gene expression by exposure to a static magnetic field in HeLaS3 cells. Cancer Res 52: 6522–6524.
10. Buemi M, \textit{et al} (2001) Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. Nepron 87: 269–273.
11. Dini L and Abbro L (2005) Bioeffects of moderate-intensity static magnetic fields on cell cultures. Micron 36: 195–217.
12. Gamboa OL, \textit{et al} (2007) Absence of relevant effects of 5 mT static magnetic field on morphology, orientation and growth of a rat Schwann cell line in culture. Histology and Histopathology 22: 777–780.
13. Pacini S, \textit{et al} (2003) Effects of 0.2 T static magnetic field on...
human skin fibroblasts. Cancer Detect Prev 27: 327–332.

14. Pagliara P, et al (2005) Differentiation of monocyctic U937 cells under static magnetic field exposure. Eur J Histochem 49: 75–86.

15. Pate K, et al (2003) Morphological evaluation of MRC-5 fibroblasts after stimulation with static magnetic field and pulsating electromagnetic field. Biomed Sci Instrum 39: 460–465.

16. Teodori L, et al (2002) Static magnetic fields affect calcium fluxes and inhibit stress-induced apoptosis in human glioblastoma cells. Cytometry 49: 143–149.

17. Yamaguchi H, et al (1993) Effects of seven months’ exposure to a static 0.2 T magnetic field on growth and glycocytic activity of human gingival fibroblasts. Biochim Biophys Acta 1156: 302–306.

18. Martino CF, et al (2010) Effects of Weak Static Magnetic Fields on Endothelial Cells. Bioelectromagnetics 31: 296–301.

19. Saunders R (2005) Static magnetic fields: animal studies. Progress in Biophysics & Molecular Biology 87: 225–239.

20. Sakashita T, et al (2010) Radiation Biology of Caenorhabditis elegans: Germ Cell Response, Aging and Behavior. J Radiat Res 51: 107–121.

21. Kawakami S, et al (2006) Effects of strong static magnetic fields on amphibian development and gene expression. Japanese J Appl Phys Part 1-Regular Papers Brief Communications & Review Papers 45: 6055–6056.

22. Kimura T, et al (2008) The effect of high strength static magnetic fields and ionizing radiation on gene expression and DNA damage in Caenorhabditis elegans. Bioelectromagnetics 29: 605–614.

23. Miyakawa T, et al (2001) Exposure of Caenorhabditis elegans to extremely low frequency high magnetic fields induces stress responses. Bioelectromagnetics 22: 333–339.

24. Hung YC, et al (2010) Effects of static magnetic fields on the development and aging of Caenorhabditis elegans. J Exp Biol 213: 2079–2085.

25. Lee CH, Hung YC and Huang GS (2010) Static magnetic field accelerates aging and development in nematode. Commun Integr Biol 3: 528–529.

26. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

27. Pfaffl MW, et al (2002) Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30: 10.

28. Gavioli Y, Sherman Y and Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119: 493–501.

29. Sandoval GM, et al (2006) A genetic interaction between the vesicular acetylcholine transporter VACHT/UNC-17 and synaptobrevin/SNB-1 in C. elegans. Nat Neurosci 9: 599–601.

30. Glenn CF, et al (2004) Behavioral deficits during early stages of aging in Caenorhabditis elegans result from locomotory deficits possibly linked to muscle frailty. J Gerontol A Biol Sci Med Sci 59: 1251–1260.

31. Huang C, Xiong C and Kornfeld K (2004) Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. Proc Natl Acad Sci USA 101: 8084–8089.

32. Casella JF, Flanagan MD and Lin S (1981) Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change. Nature 293: 302–305.

33. Sauman I and Berry SJ (1993) Cytochalasin-D treatment triggers premature apoptosis of insect ovarian follicle and nurse cells. Int J Dev Biol 37: 441–450.

34. Yuan J, et al (1993) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75: 641–652.

35. Oike Y, et al (1999) Truncated CBP protein leads to classical Rubinstein-Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. Hum Mol Genet 8: 387–396.

36. Donadon V, et al (2009) Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. World J Gastroenterol 15: 2506–2511.

37. Drinker CK and Thomson RM (1921) Does the magnetic field accelerate aging and development in nematode. Commun Sci Med Sci 38: 225–239.

38. Feychting M (2005) Health effects of static magnetic fields—a review of the epidemiological evidence. Prog Biophys Mol Biol 87: 241–246.

39. Ding J, et al (2004) Alcohol intake and cerebral abnormalities on magnetic resonance imaging in a community-based population of middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. Stroke 35: 16–21.

40. Yamamoto T, Nagayama Y and Tamura M (2004) A blood-ocytogenation-dependent increase in blood viscosity due to a static magnetic field. Phys Med Biol 49: 3267–3277.

41. Valiron O, et al (2005) Cellulare disorders induced by high magnetic fields. J Magn Reson Imaging 22: 334–340.

42. Coletti D, et al (2007) Static magnetic fields enhance skeletal muscle differentiation in vitro by improving myoblast alignment. Cytometry A 71: 846–856.

43. Amara S, et al (2009) Effect of static magnetic field and/or cadmium in the antioxidant enzymes activity in rat heart and skeletal muscle. Gen Physiol Biophys 28: 414–419.

44. Abdelmelek H, et al (2006) Skeletal muscle HSP72 and nor- epinephrine response to static magnetic field in rat. J Neural Transm 113: 821–827.

45. Houpt TA, et al (2003) Behavioral effects of high-strength static magnetic fields on rats. J Neurosci 23: 1498–1505.

46. Houpt TA, et al (2005) Behavioral effects on rats of high strength magnetic fields generated by a resistive electromagnet. Physiol Behav 86: 379–389.

47. Houpt TA, et al (2010) Repeated exposure attenuates the behavioral response of rats to static high magnetic fields. Physiol Behav 99: 500–508.