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Human Biofield Therapy Modulates Tumor Microenvironment and Cancer Stemness in Mouse Lung Carcinoma

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Abstract

Studies have demonstrated that purported biofield therapy emitted from humans can inhibit the proliferation of cancer cells and suppress tumor growth in various cancers. We explored the effects of biofield therapy on tumor growth in the Lewis lung carcinoma and expanded mechanistic outcomes. We found biofield therapy did not inhibit tumor growth. However, the experimental (Ex) condition exposed tumors had a significantly higher percentage of necrosis (24.4 $\pm$ 6.8%) compared with that of the Control condition (6.5 $\pm$ 2.7%; $P < .02$) and cleaved caspase-3 positive cells were almost 2.3-fold higher ($P < .05$). Similarly, tumor-infiltrating lymphocytes profiling showed that CD8+/CD45+ immune cell population was significantly increased by 2.7-fold in Ex condition ($P < .01$) whereas the number of intratumoral FoxP3+/CD4+ (T-reg cells) was 30.4% lower than that of the Control group ($P = .01$), leading to a significant 3.1-fold increase in the ratio of CD8+/T-reg cells ($P < .01$). Additionally, there was a 51% lower level of strongly stained CD68+ cells ($P < .01$), 57.9% lower level of F4/80high/CD206+ (M2 macrophages; $P < .02$) and a significant 1.8-fold increase of the ratio of M1/M2 macrophages ($P < .02$). Furthermore, Ex exposure resulted in a 15% reduction of stem cell marker CD44 and a significant 33% reduction of SOX2 compared with that of the Controls ($P < .02$). The Ex group also engaged in almost 50% less movement throughout the session than the Controls. These findings suggest that exposure to purported biofields from a human is capable of enhancing cancer cell death, in part mediated through modification of the tumor microenvironment and stemness of tumor cells in mouse Lewis lung carcinoma model. Future research should focus on defining the optimal treatment duration, replication with different biofield therapists, and exploring the mechanisms of action.

Keywords

biofield, Lewis lung carcinoma, apoptosis, immune modulation, stemness

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Introduction

Biofield therapies fall under the category of what the National Center for Complementary and Integrative Health (NCCIH) calls energy therapies. The NCCIH defines energy healing therapies as follows:

> A technique that involves channeling healing energy through the hands of a practitioner into the client's body to restore a normal energy balance and, therefore, health. Energy healing therapy has been used to treat a wide variety of ailments and health problems, and it is often used with other alternative and conventional medical treatments.[1]

Specific popular biofield therapies include Therapeutic Touch, Healing Touch, Polarity Therapy, Reiki, and External Qigong.

Biofield therapies continue to expand in their popularity and are being explored as possible alternative or adjuvant

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treatments for cancer.[2] Clinical trials that have examined the effects of biofield therapies have demonstrated improvements in subjective outcomes such as pain and anxiety as well as immunological outcomes.[3,4] However, when clinical outcomes are examined as the primary endpoint, some studies have not found support for biofield therapies.[5] Although this research is generally supportive, especially in improving quality of life outcomes, there are multiple methodological challenges in conducting clinical trials of biofield therapies. However, preclinical studies using cultured cells and animal models are less subject to experimental bias and concerns with placebo effects, blinding, and expectations are less relevant. Examination of the preclinical oncology research with different biofield therapies supports that these therapies do in fact modify cellular function, tumor growth, and specific biological pathways relevant for cancer growth.[6]

Research by Gronowicz et al[6] demonstrated that Therapeutic Touch modulated DNA synthesis and human osteoblast mineralization in culture and inhibited metastasis and modulated immune responses in BALB/c mice injected with the 66c14 breast cancer cells. Multiple studies by Yan and colleagues found that external qigong inhibited activation of Akt, extracellular signal-regulated kinase 1/2, and nuclear factor-κB; induced cell-cycle arrest and apoptosis; and modulated gene expression profiles in colorectal, prostate, and lung cancer cell lines.[7-10] Interestingly, Yount et al[11] found some suggestion for a dose-response effect when cells were exposed to biofield treatment. However, there is tremendous inconsistency in dose of exposure in most prior biofield therapy research.

A previous study by our team examined whether exposure to Sean L. Harribance (SLH), a purported healer,[12-15] could modulate cancer cell growth in vitro using human and mouse non–small cell lung cancer (NSCLC) cells and in vivo using a syngeneic mouse Lewis lung carcinoma model (LLC). We also examined important biological mechanisms including inflammatory and immunological pathways. We found that human lung cancer cells exposed to SLH showed reduced cell viability and downregulation of pAkt in NSCLC cells. In two separate animal studies we observed that experimental exposure of 30 minutes per session for 5 sessions slowed the growth of mouse LLC relative to a sham control group potentially by reduction of cell proliferation, suppression of inflammation, and modulation of the immune systems.[16] However, it is not clear whether the antitumor effect of biofield therapy as delivered by SLH can be altered by differential duration of the treatment. We also noted, anecdotally, that the animals in the experimental group were less active than the controls and spent more time grouped together near the front of the compartment during the experimental sessions.

The purpose of the current mouse LLC syngeneic tumor animal study was to further explore the effects SLH could have on this animal model. In addition to replicating the prior experiments, we explored whether tumor growth would be further inhibited by increasing the experimental exposure from 30 to 60 minutes and we examined the effects of biofield therapy on cell death, stem cell markers, immune markers, and animal behavior. We proposed the following null hypothesis for this study: exposure to SLH would not be able to inhibit tumor growth or affect other local or systemic oncogenic processes and targets including tumor cell apoptosis, immune cells, and stemness of cancer cells.

Methods

All experiments were performed in accordance with the relevant guidelines and regulations by The University of Texas MD Anderson Cancer Center. All assays and measurements were conducted by research staff blinded to group assignment.

Cell Line

Mouse LLC cells were purchased from American Type Culture Collection and maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

Mice and Procedures

All animal experiments were approved by The University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee. Male C57BL/6 mice were purchased from Harlan Laboratories, fed laboratory chow diet (Harlan Laboratories) and water ad libitum, and housed at the MD Anderson animal facility. The mice were acclimatized for 3 days prior to initiation of the study. LLC cells (1×10^6) were injected into the right flanks of the mice at 6 to 8 weeks of age. When the resulting tumors were palpable and on average reached a tumor volume of 9.12 ± 1.02 mm^3, the mice were randomly assigned to a Control or Experimental (Ex) group ($n = 10$ per group). The mice were housed 5 per cage and maintained in their same groupings for their respective exposures. The two groups of mice were housed approximately 10 inches apart in the animal colony. The mice were all euthanized at the same time from the start of the study with CO_2 overdose after 5 Ex/Control sessions and when tumor size of any one mouse enrolled in the study reached the allowable size limit (<2.0 cm in diameter) according with the guidelines of the MD Anderson Institutional Animal Care and Use Committee.

Tumor volume was measured every other day and calculated accordingly.[17] At the end of the study tumors were rapidly collected from the mice, weighed, and flash-frozen in liquid nitrogen or fixed in formalin for further analysis. Terminal blood was collected via cardiac puncture, and serum was prepared and stored at −80 °C for cytokine analysis.

Experimental Groups and Procedures

Experimental Exposure. The experimental condition was previously described and a similar strategy was used in this study.[16] Briefly, SLH, a psychic and healer, conducted the experimental (Ex) condition. SLH's psychic abilities have been correlated with specific neurological changes within his right prefrontal cortex as measured by quantitative electroencephalogram and single-photon emission computerized tomography.[12,14,15] What has been called the "Harribance Configuration" is a brief gamma (30-40 Hz) pattern over the right temporofrontal regions, including a major power enhancement of around 20 Hz over the right frontocentral and temporal lobe,[14] and increased activation within the right temporal lobe and extended into the adjacent insula.

Control Exposure. The control condition was previously described and a similar strategy was used in this study.[16] Lorenzo Cohen (LC) served for the control (Control) condition. LC mimicked SLH's movements when working with the animals in order to control for exposure to a human and movement. LC is a research psychologist who has conducted extensive research in psycho-oncology and studied mind-body practices such as yoga, meditation, tai chi, and qigong. Although a yoga practitioner himself, during the exposure sessions when he was observing and mimicking SLH's movements he did not focus any thought toward the animals and simply replicated SLH's movements.

SLH and LC were in the same room at the same time approximately 15 feet apart. At this distance it is possible that the purported biofield or other emissions from SLH might influence the animals in the Control condition. However, this effect would ultimately support the null hypothesis by decreasing any group differences. For the exposures, the mice were transferred to a transparent plastic box with an opaque plastic partition with 5 mice on each side. For about half the time SLH either held both hands over the experimental cage housing the mice or placed his forehead near them as it was previously shown that the electromagnetic field (EMF) activity was especially high around his right prefrontal cortex.[18] The animals were exposed to either the Ex or Control conditions for 60 minutes at each session, for a total of 5 sessions over the course of 2 weeks.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections were placed on slides and subjected to hematoxylin and eosin staining and immunohistochemical (IHC) staining for CD68, Ki67, cleaved caspase-3, and CD44. The stained slides were scanned with Aperio AT2 bright-field slide scanner and tissue sections were quantified for IHC staining with Aperio image analysis algorithms.

Immune Profiling. Tumor and spleen immune cell profiling was performed using a published method.[19] Briefly, spleen and tumor tissues were collected and placed in plain 1× HBSS. For isolation of lymphocytes from spleens and tumors, protocols described by Bartkowiak et al[19] were used. In each sample, 1×10^6 cells were used for staining immune cell surface markers. Cells were then incubated at 4 °C for 1 hour with antibodies against mouse CD4 (BioLegend), CD3 (BD Biosciences), CD8 (BioLegend), CD19 (BD Bioscience), CD45 (BioLegend), FoxP3 (BioLegend), F4/80 (BioLegend), CD206 (BioLegend), and PDL1 (BioLegend). Subsequently, the cells were washed twice with phosphate buffered saline containing 2% fetal bovine serum and then fixed and permeabilized with FoxP3 Fix/Perm Kit (ThermoFisher Scientific). Then cells were washed twice with wash buffer and incubated with intracellular markers: Foxp3 (eBioscience), tumor necrosis factor (BioLegend), and monocyte chemoattractant protein-1 (BioLegend) for 1 hour at 4 °C. Antibodies were diluted according to the manufacturers' recommendations. All the samples were collected on a BD LSRFortessa X-20 (BD Biosciences) and analyzed using FlowJo software (FlowJo v.10).

Immunoblotting. Cytosolic extracts were prepared from tumor tissues exposed to Ex or Control conditions. Tumor tissues were digested in 2 mL tubes containing ceramic beads and 75 µL of lysis buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol). After homogenization for 2 cycles of 30 seconds with Precelly's Homogenizer (ThermoFisher Scientific), the protein concentrations were determined by the Bradford assay. Immunoblot assays were performed as per standard procedure. Briefly, equal amounts (15 µg) of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose membranes. Membranes were probed with the antibodies of CD44 (No. 357259, Cell Signaling), Sox2 (No. 14962, Cell Signaling), and β-actin (No. A5441, Sigma Chemical Company). Membrane was incubated in IRDye Secondary antibody (1:20,000, No. 925-32211, LiCOR) at room temperature for 1 hour followed by 3 washes with TBS-T. Membrane was then imaged using LiCor Odyssey scanner (LI-COR Biosciences) and bands were

quantified with Image Studio Lite software. β-actin was detected for normalization of results.

Behavioral Assessments. In order to examine behavioral changes associated with exposures, 4 separate GoPro Hero3 video cameras were mounted outside the back portion of each compartment. The cameras were angled so as to capture movement from back-to-front and side-to-side within the compartment. Two assessors rated the number of times animals crossed the center of the compartment from the front half of the box to the back and from the back to the front. Individual mice were not tracked, but the group as a whole. Video recording data were only collected at the first 2 sessions, as there was concern that the recording could interfere with the outcomes. Technical problems with the recording limited evaluation of the first session and the second half of the second session for the right compartment of the Ex group. Data are presented from the second exposure session and reported as the total number of times animals crossed from front to back and back to front by group and compartment, separating the first half from the second half of the 60-minute session.

Statistical Analysis

The Prism software program (GraphPad Software) was used to perform statistical tests (*t* test or analysis of variance). *P* values less than .05 were considered statistically significant. All data are presented as means ± standard error of the mean.

Results

The experiment was carried out as planned and lasted approximately 3 weeks. None of the animals experienced weight loss and there were no differences between groups (baseline: Ex = 20.1 ± 1.7 g, Control = 20.8 ± 1.4 g; end of treatment: Ex = 21.1 ± 1.6 g, Control = 21.0 ± 1.0 g).

Experimental Exposure Increased the Number of Cells Undergoing Apoptotic and Necrotic Cell Death but Did Not Affect the Rate of Tumor Growth. As revealed by Figure 1A, there was no difference in tumor growth over time between the Ex and Control conditions. However, despite no difference in tumor volume, 66.7% of tumors from mice in the Control group were either ulcerated or slightly ulcerated whereas the mice in the Ex group showed less aggressive tumors with only 44.4% of tumors ulcerated. Hematoxylin and eosin staining indicated that 50% (n = 5) of the mice in the Ex treatment group had more than 30% of the tumor with necrotic tissue while none of the tumors in the Control group reached that level of necrosis or cell death (Figure 1B). Quantitatively, the Ex exposure treated tumors had a significantly higher percentage (24.4 ± 6.8%) of necrosis compared with that of the Control condition (6.5 ± 2.7%; $P = .019$; Figure 1C). When the tumor tissues were stained for cleaved caspase-3, a marker of the apoptotic cell death, the percentage of total cleaved caspase-3 positive cells in Ex exposure tumor tissues was 8.1 ± 1.7%, which is 2.3-fold higher than that of the Control condition (3.5 ± 0.8%; $P < .05$; Figure 2A-E), suggesting that biofield therapy could potentially lead to increased apoptotic cell death and tumor necrosis. All tissues were examined microscopically by a certified pathologist in a blinded fashion.

Biofield Therapy Modified Tumor Immune Cell Profiles. In our previous study, Yang et al[16] reported that Ex exposure led to marked reduction in the CD68 positive staining macrophages in the tumor tissues and altered immune cell profiles in the tumor tissues. In the current study, IHC staining revealed a decrease of CD68+ macrophages in tumor tissue from the Ex group (Figure 3A). Although the level of total CD68+ macrophages, including mild (+1), moderate (+2), and marked (+3) stained cells in Ex exposure tumor tissues was moderately less than that of the Control condition, there was an approximately 51% reduction in markedly stained (3+) CD68+ cells in the Ex exposure group compared with that of the Control group ($P < .01$). Consistent with these findings, immune profiling of TILs also showed that the abundance of F4/80+/CD206+, M2 tumor-associated macrophages in the Ex exposure tumor tissues were significantly reduced in comparison to that of the Controls (Figure 3B-f; $P < .02$), with no group differences in M1 macrophages (F4/80+/CD206−; Figure 3B-e). Decreased M2 resulted in a significant 1.8-fold increased ratio of M1/M2 in the Ex exposed tumors (Figure 3B-g; $P < .01$), further suggesting that the Ex exposure suppressed tumor-associated macrophages in mouse lung carcinoma. Additionally, as we reported previously,[16] we also observed significantly higher CD8+ cytotoxic T-cells ($P = .007$; Figure 3B-a) and significantly lower FOXP3+ T-reg cells ($P = .001$; Figure 3B-b) as well as significantly higher ratio of CD8+/FOXP3+ cells ($P < .002$; Figure 3B-c) in Ex exposure tumor tissues compared with that of the Control group. The level of tumor necrosis factor-α in CD8+ cells of the Ex group was also lower than that of the Control group (Figure 3B-d), but the reduction did not reach statistical significance. Taken together, these data suggest that the Ex exposure enhanced antitumor immunity.

Biofield Therapy Significantly Downregulated the Expression of the Stem Cell Marker SOX2 in LLC Tumor Tissues. To examine the influence of the Ex exposure on LLC stemness, we measured the protein expression of CD44 and SOX2 in the LLC mouse tumor tissues. The IHC staining clearly indicated that the CD44 expression was markedly reduced in Ex-exposed tumor tissues compared with that of Control (Figure 4A). Although not statically significant, the relative CD44 expression was decreased in the Ex condition tumor tissues as assessed by western blot with a 15% reduction in the Ex condition (Figure 4B). Intriguingly, Figure 4C reveals a significant 33% decrease of SOX2 protein, another

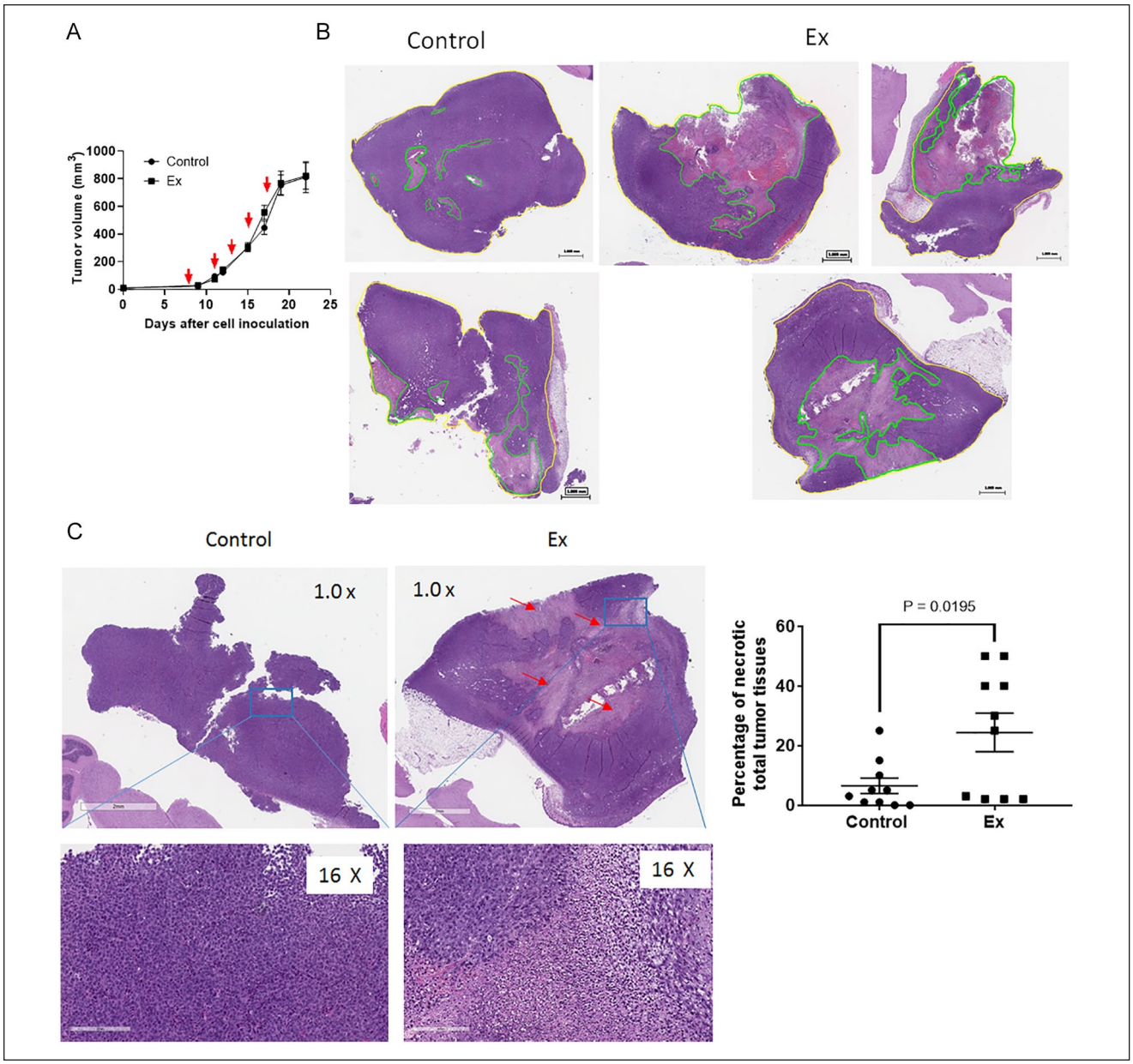

Figure 1. The effect of Ex exposure on tumor growth in a mouse LLC model. (A) The tumor growth curves for Ex (n = 10) and Control mice (n = 10) with LLC in which treatment started when tumors were palpable (approximately 10 mm^3). The red arrow indicates when treatment was administered. (B) H&E staining of LLC tumor with necrotic tumor tissues in Control and Ex conditions. Green lines delineate the necrotic tissues of tumors on H&E-stained histologic sections. (C) LLC tumor exposed to Ex had significantly higher number of necrotic cells than that of the Control group. Data are presented as means ± standard error.

marker of cancer stemness, in the Ex condition compared with that of Control ($P < .02$).

Behavioral Observations. Although exploratory in nature, we observed marked differences in the behavior of the mice with Ex versus Control condition during the second 60-minute treatment session. For both groups, exploratory behavior was higher for the first 30 minutes versus the second 30 minutes, but the Ex group engaged in almost 50% less movement throughout. While the mice in the Control condition tended to more consistently explore their environment throughout the 60-minute exposure, the mice in the Ex condition tended to cluster together for a greater amount of the time with less movement from front to back and back to front of the compartment (Figure 5). Due to technical difficulties, we did not have a recording for the Ex exposure group, right compartment for the second 30 minutes of recording. However, an interesting observation is that

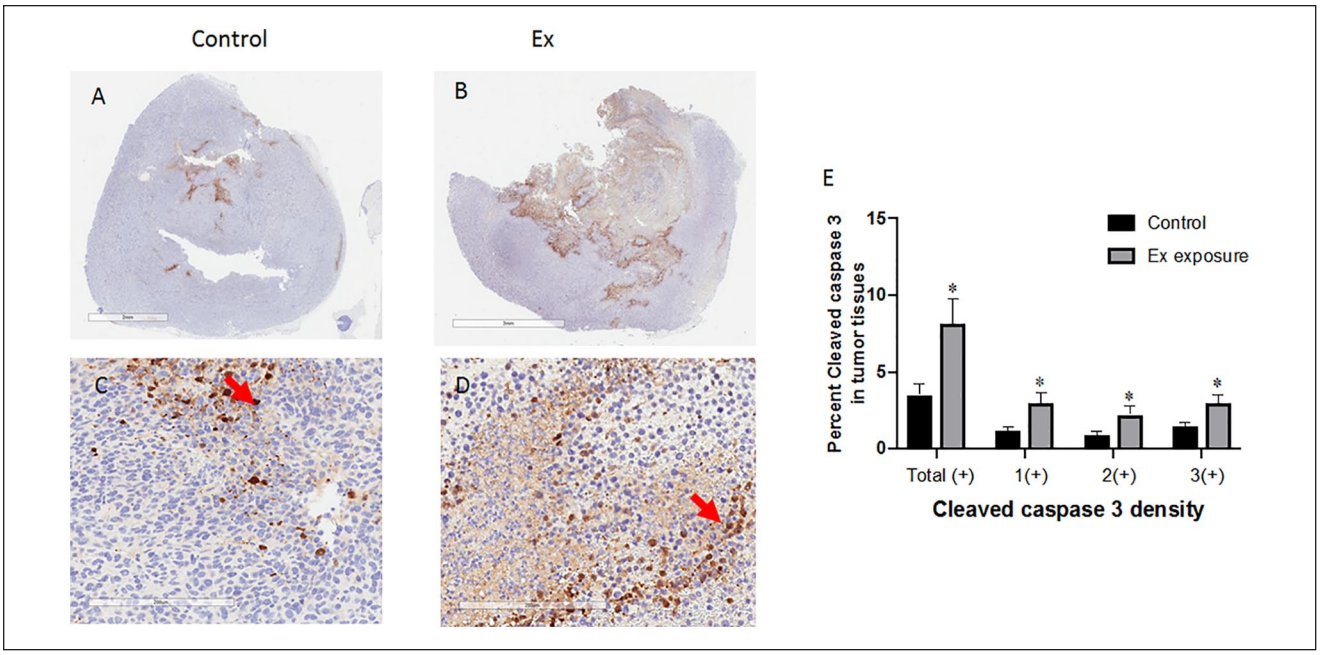

Figure 2. IHC staining of cleaved caspase-3 protein in LLC tumor tissues of the Control group (A and C) and Ex group (B and D). (E) Quantitative analysis of cleaved caspase-3 positive staining in tumor tissues. (A and B) Images were taken at 1× magnification, and C & D images were obtained at 20× magnification with ImageScope on Aperio AT2 scanned slides. Red arrows refer to cleaved caspase-3 positive staining cells. Data are presented as mean ± SE. *$P < .05$ versus Control group.

although the Ex exposure group mice tended to have a similar amount of movement during the first 30 minutes, the mice in the left compartment tended to group together right at the front of the compartment and the mice on the right side tended to group near the back. Further examination of the necrosis levels of the mice from Figure 1C revealed that the 3 mice in the Ex condition with no necrosis happened to be housed in the right compartment.

Discussion

The current study did not reject the null hypothesis in terms of tumor growth, as the groups did not differ in tumor volume during the testing period. However, the Ex exposure resulted in an increase in apoptosis and necrotic cell death and modulation of multiple oncogenic processes including decrease in the markers of the stem cells and modulation in immune cells. Increase in apoptosis and modulation of TILs along with decrease in stem cell markers are all relevant for inhibiting the growth and metastasis of tumors. The Ex exposure was not associated with any noted toxicities, as there was no weight loss in any of the animals.

In our prior study, we found that 30 minutes of exposure for 5 times to biofield therapy significantly inhibited the growth of LLC tumor when the treatment was started when the tumor volumes were just palpable.[16] In this current study, we also initiated the treatment when the tumor size was relatively small (<10 mm^3). However, we did not observe group differences in tumor volume between Control and Ex exposure conditions when these mice were exposed to 60 minutes of treatment per session for 5 sessions. The lack of group differences in tumor volume in the current study might be due to the higher percentage of necrotic tissues in the Ex exposure group compared with that of Control condition. The larger amount of necrotic tissues can be either generated due to higher tumor growth rate or it may occur when the death rate of cells exceeds the ability of the cells to clear dying cells, which could be caused by apoptosis, autophaghy, or necrosis.[20] The growth rate of tumor in both Ex exposure condition and Control condition was similar (Figure 1A). Thus, larger amounts of necrotic tissues in Ex exposure group is likely due to the higher number of dead cells induced by longer exposure (60 minutes vs 30 minutes) to biofield therapy. Indeed, in the current study, with 60-minute exposure to biofield therapy, there was a higher number of apoptotic cells as evidenced by the significantly higher positive staining of cleaved caspase-3 in Ex exposure condition than that of Control condition (Figure 3) whereas as in the prior study, with 30 minutes exposure, there was no statistically significant group difference on cleaved caspase-3 positive staining cells. Additionally, the stronger staining of cleaved caspase-3 positive cells appeared to be predominantly localized at the edge of the necrotic tissues near the area of transition to live tumor cells (Figure 3D). It is well-documented that the necrosis or necrotic tissues leads to secretion of intracellular proteins that activate the damage

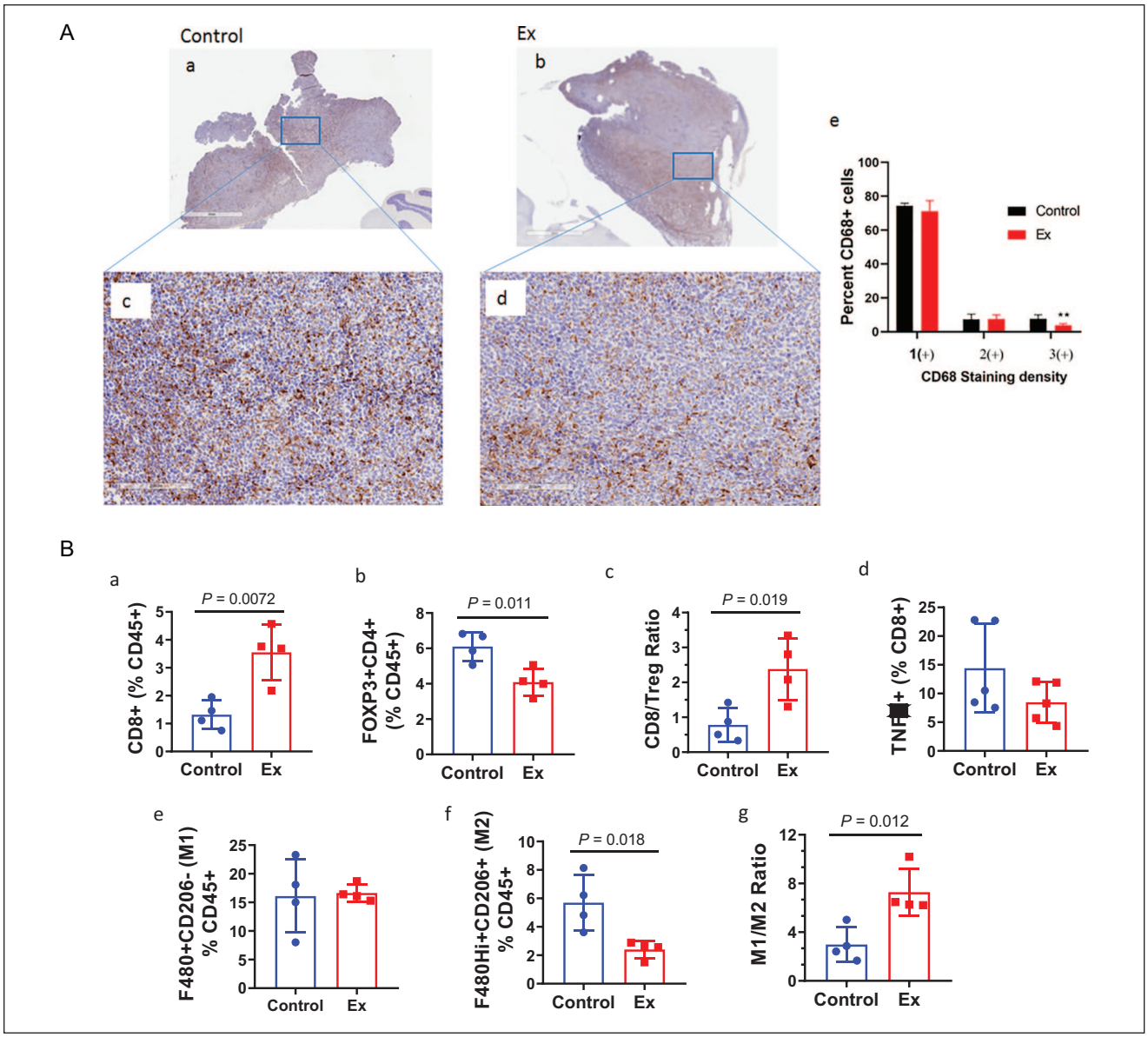

Figure 3. Immune modulation within the tumors of mice with LLC. (A) CD68 positive staining of tumor-infiltrating macrophages from Controls (a and c) and Ex condition mice (b and d). (e) Quantification of CD68 positive cells in tumors of mice with LLC. (a and b) were taken at 1× magnification and (c and d) at 20×. Data are presented as mean ± SE. $^{**}P < .01$ versus Control. (B) Immune cell profiling in LCC tumor exposed to Control or Ex conditions. The immune cells staining with surface markers of (a) CD8; (b) FoxP3; (c) ratio of CD8 over FoxP3; (d) TNF-α; (e) F4/80 (+)/Cd206 (−); (f) F4/80 (+)/CD206 (+); and (g) ratio of M1/M2 macrophages. Data are presented as mean ± SE.

response (DAMPs), which stimulates inflammatory response and immune amplification.[21] In fact, we did observe that levels of serum cytokines including interleukin-6 and MCP-1 were higher in mice exposed to the Ex condition than that of the Control conditions (data not shown). Elevated cytokine levels and DAMPs could potentially lead to the growth of tumor[22] and attenuate the antitumor activity of Ex exposure as shown in our previous study.[16] Further identification of the key factors in the tumor tissues under longer Ex exposure condition will help identify the cause of lack of overall growth inhibition of the LLC tumor.

In light of recent studies with immune checkpoint inhibitors, finding no group differences in tumor growth may be expected. Research with immune checkpoint inhibitors show that these drugs cause immune cells to infiltrate into the tumor microenvironment.[23] Early in the treatment process this can cause tumors to grow leading to a clinical indication that the treatment is not working. However, the

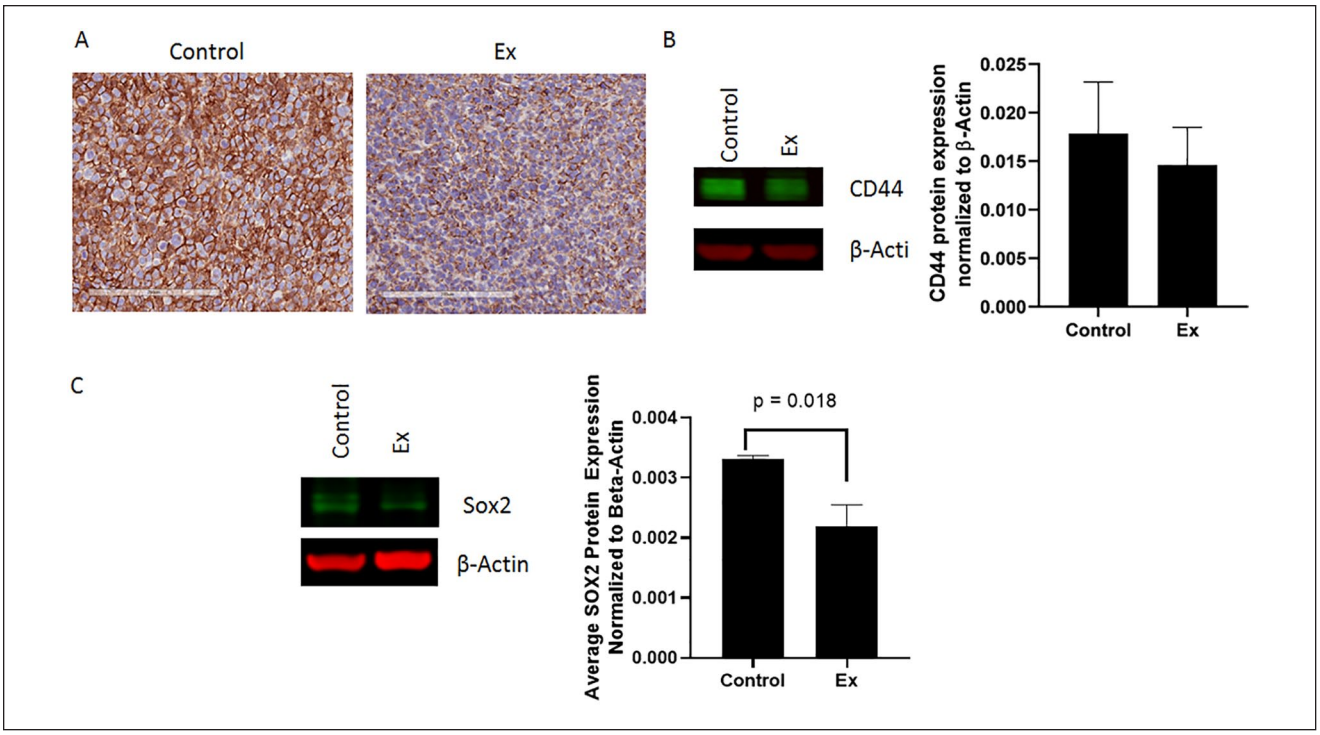

Figure 4. (A) Ex exposure inhibited the expression of stem cell markers in LLC tumor tissues. IHC staining of CD44 in the tumor tissues. Pictures were taken at 20× magnification. (B) Protein expression of CD44 in LLC tumor tissues from Control- and Ex-treated mice by western blotting. (C) Protein expression of SOX2 in LLC tumor tissues from Control and Ex condition by western blotting. Data are presented as mean ± SE.

enlargement of a tumor is partially caused by the increased numbers of infiltrating immune cells and accumulation of necrotic tissue.[23] In the current study, we found a similar effect with an increase in TILs, especially CD8+ T-cells (Figure 3B-a), M2 macrophages (Figure 3B-f), and necrotic cells. This may also help explain why there was no difference in tumor size and weight by the end of the study. If the trial would have continued for a longer period, it is likely that the tumors with more necrosis would have shrunk more than those with less necrotic tissue. In the prior study, we also found a down regulation of PD-L1 in the tumor microenvironment.

Even though we failed to observe the overall tumor suppression by the Ex exposure in mice bearing LLC tumor, our data continue to suggest that biofield therapy can alter the tumor microenvironment, such as immune modulation, and directly targets tumor cells, especially cancer stem cells (CSCs), which may have a tumor suppressive role. Accumulating evidence suggests that only a small population of cells within a tumor mass are responsible for tumor heterogeneity. Most of these cells are also considered CSCs and are characterized by their ability to self-renew and differentiate into multiple cell types. CSCs are thus responsible for tumor initiation, progression, recurrence, and metastasis.[24,25] Therapies specifically targeting CSCs hold great promise for improving survival outcomes in patients with cancer, including lung cancer. Mounting evidence suggests the markers such as CD44 and SOX2 are critical regulators of cancer stemness, including self-renewal, tumor initiation, and metastasis.[26-28] SOX2 was frequently overexpressed and essential for maintenance of NSCLC cells to reinitiate and drive tumorigenesis.[29-31] It is remarkable that biofield therapy was capable of significantly inhibiting the expression of stem cell markers, especially SOX2, in mouse LLC (Figure 4B). It was speculated that electromagnetic energy emission may potential affect the stem cell fate.[32] However, whether biofield therapy is able to alter the stemness of cancer cells has yet to be studied. In light of the critical role of SOX2 in cancer cell stemness, our study for the first time demonstrates that biofield therapy may contribute to reducing the stemness of cancer cells, which deserves further investigation.

There were clear differences in the behavior of the 2 groups, with the experimental group showing less activity during the 60-minute session. The animals in the experimental group tended to remain together and engaged in less exploratory behavior. Of note, the experimental mice in the left chamber tended to remain near the front of the chamber to a greater extent whereas those on the right side tended to

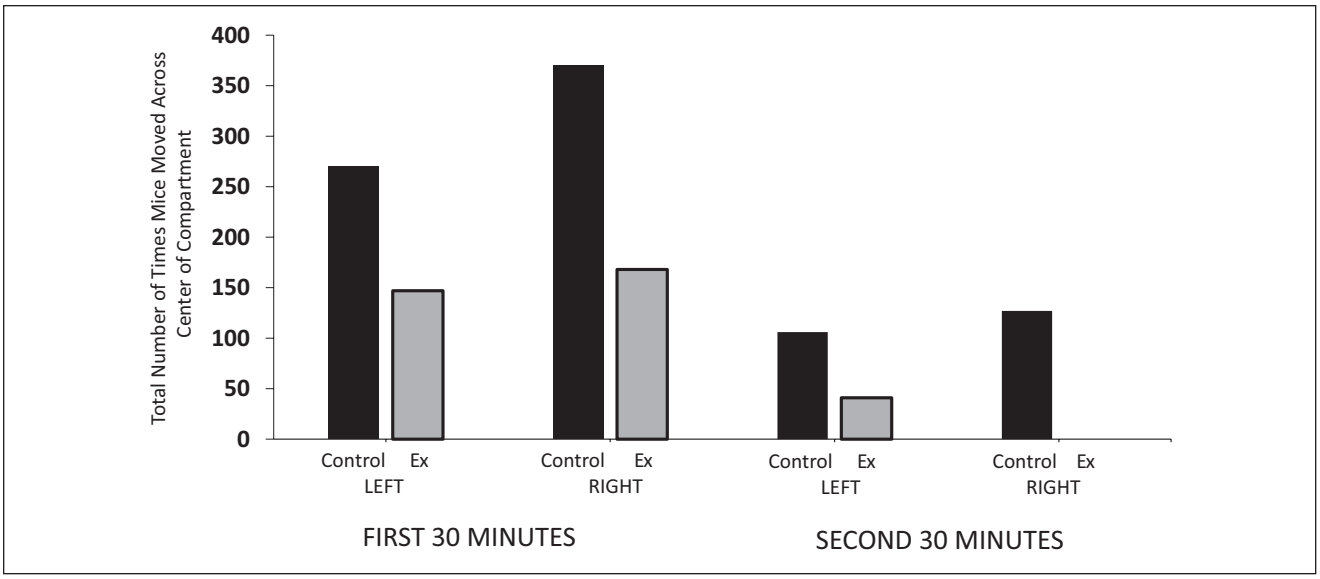

Figure 5. Behavioral observations by group and compartment for the first and second 30 minutes of the second 60-minute exposure session. Data are reported from the second 60-minute exposure session and presented as the number of times animals crossed from front to back and back to front by group and compartment for the first and second 30 minutes of the session. Due to technical difficulties, we did not have a recording for the Ex group, right compartment for the second 30 minutes of recording.

remain together near the back of the chamber. It is interesting that the mice on the left compartment had the greatest levels of necrosis and 3 mice on the right were not that dissimilar to the control mice. The behavioral observations correlating with the clinical changes may be related to possible EMF emissions from SLH, as it was previously shown that EMF activity was especially high around his right prefrontal cortex.[18] However, this remains speculation, as EMFs were not measured in the current study. Additionally, the mice in the experimental group were clearly "calmer." This suggests another plausible mechanism could be through modulation of the sympathetic nervous system. Exposure to a "healer" could result in a calming effect that could lead to beneficial changes in the tumor microenvironment.[33] In order to better understand the mechanisms whereby humans may affect tumor growth, it is critical that future studies measure the purported emissions from the body, conduct experiments to manipulate said emissions (eg, blocking or enhancing experiments), and conduct more in-depth studies of the animals.

There are some limitations in this study. Most important, we did not measure any of the purported mechanisms associated with this form of treatment such as EMFs, biophoton emissions, or other mechanisms of action. The behavioral data were also collected in an exploratory fashion and the rating of activity was not conducted using a validated technique. However, the differences in the activity between groups were substantial, reducing some concern over using a non-validated technique. Regardless, we recommend that future research should use a validated technique and animal chambers with appropriate equipment for objective scoring of animal activity. Some concern exists when using electronic equipment that could interfere with the treatment effect. Although our study was relatively small, the results are consistent with our prior studies and others showing that biofield therapies can alter the tumor microenvironment and systemic function in in vivo cancer models. We did not conduct in-depth measures of toxicities in this study, but the lack of weight loss associated with Ex exposure suggests no toxicity, which is in contrast with conventional chemotherapies, radiotherapy, immunotherapy, or other targeted therapies.

Summary

Altogether, these results indicated that experimental exposure to biofield therapy induced increased cell death as assessed by necrosis and apoptotic cell, perhaps through modulation of immune pathways and modification of the tumor microenvironment and stemness of tumors of mice with lung carcinoma. Further research should focus on defining the optimal treatment duration/dose, replicating the effect with different biofield therapists, and exploring the mechanisms of action of biofield therapy.

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Author Contributions

PY, SLH, and LC designed the research; PY, PRR, TC, SN, MG, SLH, and LC performed the experiments; PY, TC, SN, VH, and MG analyzed the data; PY, TC, SLH, MG, JS, and LC wrote and reviewed the manuscript. All authors approved and contributed to the manuscript at various stages.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: SLH has a private practice in Sugar Land, Texas, and is the president of the Sean Harribance Institute for Parapsychology, Inc. He is also the honorary director of the Sean Harribance Institute for Parapsychology Research, a 501(c)(3) corporation. None of the other authors declared any conflicts of interest.

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