Human biofield therapy does not affect tumor size but modulates immune responses in a mouse model for breast cancer

Gloria Gronowicz¹, Eric R. Secor Jr.¹,², John R. Flynn¹, Liisa T. Kuhn⁴

¹. Department of Surgery, University of Connecticut Health Center, Farmington, CT 06030, USA
². Department of Immunology, University of Connecticut Health Center, Farmington, CT 06030, USA
³. Hartford Healthcare, Hartford Hospital, Hartford, CT 06102-5037, USA
⁴. Department of Reconstructive Sciences, University of Connecticut Health Center, Farmington, CT 06030, USA

ABSTRACT

OBJECTIVE: To assess the effect of human biofield therapy, an integrative medicine modality, on the development of tumors and metastasis, and immune function in a mouse breast cancer model.

METHODS: Mice were injected with 66cl4 mammary carcinoma cells. In study one, mice received biofield therapy after cell injection. In study two, mice were treated by the biofield practitioner only prior to cell injection. Both studies had two control groups of mock biofield treatments and phosphate-buffered saline injection. Mice were weighed and tumor volume was determined. Blood samples were collected and 32 serum cytokine/chemokine markers were measured. Spleens/popliteal lymph nodes were isolated and dissociated for fluorescent-activated cell sorting (FACS) analysis of immune cells or metastasis assays in cell culture.

RESULTS: No significant differences were found in weight, tumor size or metastasis. Significant effects were found in the immune responses in study one but no additional effects were found in study two. In study one, human biofield treatment significantly reduced percentage of CD4⁺CD44loCD25⁺ and percentage of CD8⁺ cells, elevated by cancer in the lymph nodes, to control levels determined by FACS analysis. In the spleen, only CD11b⁺ macrophages were increased with cancer, and human biofield therapy significantly reduced them. Of 11 cytokines elevated by cancer, only interferon-γ, interleukin-1, monokine induced by interferon-γ, interleukin-2 and macrophage inflammatory protein-2 were significantly reduced to control levels with human biofield therapy.

CONCLUSION: Human biofield therapy had no significant effect on tumor size or metastasis but produced significant effects on immune responses apparent in the down-regulation of specific lymphocytes and serum cytokines in a mouse breast cancer model.

Keywords: biological therapy; breast cancer; immune system; integrative medicine; cytokine; energy medicine

1 Introduction

1.1 Breast cancer

One of the most common forms of cancer in women in the world is breast cancer. Each year in the United States, more than 200,000 women develop breast cancer and more than 40,000 women die from the disease. Men are also diagnosed with breast cancer but it is less common in men—approximately 2,000 men per year are diagnosed with breast cancer and 400 men will die from the disease. The majority of cancer patients use integrative medicine to help relieve symptoms from cancer and its treatment for it: such as fatigue, loss of appetite, difficulty with sleeping and eating, stress, and depleted immune system[1,2,3]. Although energy medicine is a small part of these integrative medicine therapies, a recent study demonstrated that cancer patients reported the highest benefit ($P<0.004$) with energy medicine compared to other integrative medicine therapies[4]. Evidence-based research is needed to determine the efficacy of these therapies. Therefore, the aim of this study was to assess the effect of energy medicine in a preclinical model of breast cancer in mice. Our hypothesis was that energy medicine would have effects on tumor growth, metastasis and immune function.

1.2 Energy medicine

Energy medicine is one of the domains in integrative medicine that is focused on the human biofield being able to promote well-being and health. The concept of a human biofield in energy medicine has its origins in many different ancient cultures with the development of numerous types of biofield therapies—Reiki, external Qi therapy, Healing touch, Therapeutic touch, etc., but only recently has Western science begun evaluating the potential of these therapies. Energy medicine has a substantial history of clinical trials that reflects the safety of this intervention and some beneficial therapeutic effects. For example, in clinical studies with a particular energy medicine modality, Therapeutic touch, this energy medicine modality was shown to decrease anxiety of patients in various clinical settings[5,6], decrease pain[7,8], diminish anxiety and pain[9,10], improve functional ability in patients with arthritis[11,12], enhance personal well-being[13], and facilitate rest/sleep[14,15,16]. In addition, energy medicine treatments had positive effects on the immune system in patients with stress and anxiety[11,17], and on hematocrits and hemoglobin in human subjects[18]. Particular well-known healers have been recruited to treat cell cultures and have shown significant effects[19–22], while other studies have used groups of practitioners to elicit significant effects[23–28]. Since energy medicine is practiced on individuals without known medical issues, it was also important to determine if pretreatment with human biofield therapy on subjects prior to a procedure or health issue would have an effect. Therefore, the second arm of our study was undertaken with pretreatment.

1.3 Mechanism

The mechanism for human biofield therapies is not known but electromagnetic fields are considered to be a component[30,31]. In biology, electromagnetic fields emitted by the heart and brain, and other organs are well-accepted. Medicine and science measure the pattern of these electromagnetic biofields to monitor the health of the heart and brain through electrocardiograms, electroencephalograms and magnetoencephalograms. Pulsed electromagnetic fields (EMFs) have been shown to inhibit tumor growth and tumor angiogenesis in animals[32,33]. In a breast cancer model in mice, EMFs significantly reduced tumor growth and the extent of vascularization with increased tumor necrosis in animals[34]. There have also been reports that continuous exposure to EMF can enhance the growth rates of transformed cells in culture for some human epithelial cancers[35]. EMFs have been shown to enhance the effects of chemotherapy[36,37]. In human biofield studies, very low EMFs have been detected from the hands of practitioners[38–40]. Therefore, the effect of energy medicine on other living creatures, such as mice, being partly due to EMFs might not be unexpected. In other studies, forces outside of the electromagnetic spectrum have been considered a component of the human biofield[41].

1.4 Rationale for study design

A breast cancer model in mice derived from an aggressive 4T1 mouse mammary carcinoma was used to study the effect of energy medicine on cancer[42]. Breast cancer tumors develop after 66cl4 cells are injected into the mouse. This cell line is a thioguanine-resistant variant of line 66 and these cells are able to metastasize into lymph nodes. Isolation and culture of cells from the lymph nodes in the presence of 6-thioguanine allows the survival of metastasized 66cl4 cells, which grow in colonies that can be counted as an index of metastasis. The growth of the tumor depends on the secretion of growth factors, cytokines/chemokines to enhance cell proliferation and invasiveness, angiogenesis, leading to inflammation and recruitment of immune cells including macrophages, which in turn secrete additional cytokines. During metastasis, cells and factors eventually migrate into other tissues through the blood stream and can be found in organs such as the spleen. To survey for any major changes in serum cytokine/chemokine markers, thirty-two cytokines were screened using a commercially available kit. A broad spectrum of lymphocytes and macrophages were also analyzed in the spleen and lymph nodes by fluorescent-activated cell sorting (FACS) to identify major changes in immune cells. This study is
the first to examine in depth the effect of human biofield therapy on the immune system in breast cancer.

2 Materials and methods

2.1 Cells

The 6-thioguanine-resistant 66cl4 cell line was derived from an aggressive 4T1 mouse mammary carcinoma and metastasized to popliteal lymph nodes, draining from the primary tumor, where colony formation can be determined\cite{42}. The 66cl4 breast cancer cells were cultured from frozen stocks approximately 2 weeks prior to cell injections in 175 cm\textsuperscript{2} flasks with RPMI-1640 medium (with 2 mmol/L L-glutamine, 1% pyruvate and 1 mmol/L non-essential amino acids), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.

2.2 Mice

Tumor-free, 6–8 week old, female BALB/c mice (Charles River, NCI) were received one week before study onset so that mice could acclimate. All experiments were approved by the Animal Care Committee of the University of Connecticut Health Center. Twenty microliters of a 3.6 × 10\textsuperscript{7} cells/mL cancer cell suspension (66cl4 in phosphate-buffered saline (PBS)) were injected into the right rear footpad of the mice to initiate tumors on the same day for each experiment. Four or five mice were housed per cage, and in the first study each treatment group consisted of 16 mice. An additional group of eight mice received 20 \mu L vehicle/PBS injections, and was considered the negative control group. For the second study, twelve mice per treatment group were used, as well as an additional eight mice for the negative control. Mice were assigned to groups in a random manner. At least twice a week mice were visually examined to determine the extent of tumor formation. Approximately four weeks after cell injections all mice had large tumors and mice were euthanized. All mice were weighed at the start of the experiment and at the end. Euthanasia was performed on the same day for each experiment. Spleen and popliteal lymph nodes were isolated and blood was obtained by cardiac puncture.

2.3 Treatments

In the first study, two and twenty-one days after the cancer cell injections, an internationally recognized healer\cite{39,44}, treated sixteen mice. Each treatment lasted approximately five minutes and consisted of the practitioner sitting two feet away from the mice on a low bench with the tops of their cages open (four mice per cage), and performing a technique of mental energy transmission (mice were labeled TR). In the first treatment, two authors observed that the mice lay down in their cages while being treated. This was repeated to a lesser extent in subsequent treatments. The methodology stated by the practitioner was “to focus the mental energies into mentally connecting the target to what was seen by the practitioner as a larger field of intelligent energy and to then channel the energy into the targets.” Another set of 16 mice with cancer received mock treatments in the same location with their cages open for the same amount of time on another day and with a technician in same proximity to the mice (mice were labeled CA). The mock treatments were administered by different individuals in the laboratory, who had no training in any biofield technique and followed a similar protocol. To remove intention, this person counted backwards from 1000. The third group of eight mice was PBS-injected mice and received no treatments (mice were labeled PBS).

On the 26th day after the injections, all mice had large tumors in their right footpad and were euthanized. After the completion of the first study, a second study was undertaken in which mice were treated by the biofield practitioner prior to having cancer cells injected to determine if energy medicine would have any effect on the onset or progression of cancer. Twelve mice were treated for approximately 5 min, and then two weeks later the mice were treated again in a similar manner by the same individual (TR2). Another set of 12 mice, received mock treatments in the same location with their cages open on another day for 5 min (CA2). Twenty-four hours after the last treatment by the practitioner, all 24 mice received 20 \mu L injections of the cancer cells (3.7 × 10\textsuperscript{6} 66cl4 cells/mL) into their right rear footpad. Another group of eight mice were PBS-injected (PBS2). No other biofield or mock treatment was given after cell injections. Mice were weighed at the beginning and end of the experiments, and checked twice a week for tumor growth. On the 29th day after injections when tumors were large in all mice, the mice were weighed and euthanized.

2.4 Tumor volume measurements

The length and width of the tumors were measured with a caliper. The size of the tumor was determined by the formula: tumor volume = \frac{1}{2} (length × width)^{2}\cite{45}.

2.5 Flow cytometry

From each group of eight mice, the spleens and popliteal lymph nodes were harvested, mechanically disrupted, and passed through a 70-\mu m nylon cell strainer (BD, Bedford, MA, USA) to produce a single-cell suspension. Erythrocytes were lysed in each spleen suspension by rinsing with deionized H\textsubscript{2}O at room temperature for 15 s. Lysis was terminated by the addition of Hank’s balanced salt solution (Sigma). Both popliteal and spleen cells were washed in FACS Buffer (PBS containing 0.2% bovine serum albumin and 0.1% NaN\textsubscript{3}) and aliquots of 10\textsuperscript{6} cells.
were incubated with 100 µL of appropriately diluted antibodies for 30 min at 4 °C. The following monoclonal antibodies were used for mouse cellular surface staining: CD44 (eFluor 450), CD49b (FITC), CD4 (APC), CD8a (PerCP-eFluor 710), CD11b (PE-Cy7), CD19 (APC-eFluor 780), CD25 (PE) and were purchased from eBioscience (San Diego, CA, USA). Cells were fixed with 4% paraformaldehyde in PBS. Relative fluorescence intensities were determined on a 4-decade log scale by flow cytometric analysis, using an LSRII (Becton Dickinson, San Jose, CA, USA). Five hundred thousand cell events were collected per sample. Analysis was carried out with FACSDiva software (BD Biosciences, San Jose, CA, USA). These analyses were performed by the FACS facility at the University of Connecticut Health Center without knowledge of sample identity.

2.6 Serum cytokine assay
Peripheral blood was collected via cardiac puncture, allowed to clot for at least 30 min and then centrifuged. Serum aliquots were frozen at −80 °C until assayed. Serum cytokine levels were determined with the Milliplex mouse cytokine/chemokine magnetic bead premixed 32plex kit (MCYTMAG-70K-PX32, Millipore Co, Billerica, MA, USA) according to the manufacturer’s protocol. This assay was performed by the Clinical Research Center at the University of Connecticut Health Center without knowledge of sample identity.

2.7 Metastasis assay
The popliteal nodes on the tumor side and on the non-tumor side were collected from mice in each treatment group. The node on the non-tumor side served as a negative control (C). Each node was placed into one well of a six-well plate with 3 mL of culture medium consisting of 60 mmol/L 6-thioguanine in RPMI-1640, 1 mmol/L non-essential amino acids, 2 mmol/L L-glutamine, 100 Units/mL penicillin-streptomycin, 1% pyruvate and 10% FBS. Each node was dissociated and incubated for 12 d in medium. The medium was removed and the cells were fixed with methanol and stained with 0.03% w/v methylene blue. Cells were rinsed with water and allowed to dry. Without knowledge of treatment groups, the number of colonies/well was counted in the light microscope by two independent observers.

2.8 Statistics
Results were obtained from eight mice per group for the first study and six mice per group for the second study and represented as mean ± standard error or standard deviation, as specified. The statistical analysis was performed by analysis of variance (ANOVA) with multiple comparison procedures to isolate group mean differences followed by the Bonferroni posttest with the conventional P<0.05 level considered statistically significant.

3 Results

3.1 Weight and tumor volume
Mice in all groups gained weight and survived the treatment (Table 1). There were no significant differences in weight gain between any of the groups. Measurements of tumor volume also revealed no significant differences between groups (Table 1). Histological analysis of paraffin-embedded tumors demonstrated no significant histological differences between groups (data not shown). Immunohistochemistry also revealed the same percentages of apoptotic and proliferating cells.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Weight 1 (g)</th>
<th>Weight 2 (g)</th>
<th>Tumor volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>18.1 ± 0.3</td>
<td>19.8 ± 0.3</td>
<td>0</td>
</tr>
<tr>
<td>CA</td>
<td>17.7 ± 0.3</td>
<td>19.2 ± 0.3</td>
<td>222.6 ± 18.1</td>
</tr>
<tr>
<td>TR</td>
<td>18.4 ± 0.1</td>
<td>19.8 ± 0.2</td>
<td>282.0 ± 27.8</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>20.3 ± 0.3</td>
<td>21.4 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td>CA</td>
<td>20.7 ± 0.2</td>
<td>21.4 ± 0.5</td>
<td>237.6 ± 18.5</td>
</tr>
<tr>
<td>TR</td>
<td>20.8 ± 0.2</td>
<td>21.1 ± 0.3</td>
<td>251.8 ± 36.2</td>
</tr>
</tbody>
</table>

Table 1 Changes in mouse weight and tumor volume

There were no significant differences in the weights between PBS and the treatment groups either at the start of the experiments (Weight 1) or at the end (Weight 2). Mice injected with breast cancer cells (CA and TR) increased their weights during the treatment periods in a similar manner as the PBS-injected mice. Tumor volumes were not significantly different between CA and TR in either experiment. Values are mean ± standard deviation. PBS: phosphate-buffered saline.
of metastasis by colony formation demonstrated that most mice had a range from two to nine colonies with no significant difference between the groups. In both CA and TR groups, there was one out of eight mice that had one or no metastasis to the popliteal lymph nodes and one mouse in both groups that had more than ten colonies. In the contralateral limb without cancer cell injection (C), no tumors developed by visual inspection, and no metastatic colonies were found after culture of the popliteal node.

3.3 Cytokines

Analysis of 32 cytokine/chemokine markers in the serum revealed a change in the levels of 11 markers in the mice with cancer cell injections (CA) compared to PBS-treated mice (PBS). There was an increase in the concentration of interferon-gamma (IFN-γ), interleukin (IL)-2, IL-4, IL-5, IL-12 (p40), IL-1α, IL-1β, IFN-γ-induced protein 10 (IP-10), macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein-2-alpha (MIP-2α), and monokine induced by IFN-γ (MIG) in the serum of CA mice compared to PBS mice. The other 21 cytokines were either not detected or were not significantly altered by cancer.

In the second study, changes in the same group of 32 cytokine/chemokine markers were analyzed. Of the 11 markers that had previously shown significant differences between the PBS2 mice and the CA2 mice, seven significantly changed again in the same manner: IL-1α, IL-1β, IFN-γ, MIP-2α, IL-2, MIG, and IP-10. Another two cytokines from the same group of 11 cytokines also changed but instead of finding an increase of IL-5 and IL-12 (p40) with cancer, they were significantly decreased in the serum of CA2 mice compared to PBS2 mice. No significant differences were found with IL-4 or M-CSF. Therefore, changes in cytokines with cancer were mostly reproducible in this mouse model of breast cancer.

Analysis of only the 11 cytokine markers that changed with cancer, demonstrated that levels of five cytokines significantly decreased to PBS levels with human biofield treatment (TR) compared to the serum from mice that had cancer and mock treatment (CA): MIP-2α, MIG, IL-1β, IL-2, and IFN-γ (Figure 2). Thus, half of the cytokines altered by cancer were down-regulated to normal levels by human biofield treatment.

In the second study involving only pretreatment with the human biofield before cell injections, the practitioner did not elicit any significant effects on serum cytokine markers or specifically labeled lymphocytes analyzed by FACS. Therefore, human biofield treatment had no significant effects on them if mice were treated by the biofield practitioner only prior to the development of cancer.

3.4 FACS

In the first study, FACS analysis of specifically labeled lymphocytes revealed changes with cancer only in the popliteal lymph nodes. In the popliteal lymph nodes, the following lymphocytes were significantly elevated with cancer: percentage of CD45+CD44hiCD25−, percentage of CD44hiCD25−, percentage of CD44loCD25−, percentage of CD19+ (B cells), and percentage of CD8+ T cells. The percentage of CD11b+ macrophages was also elevated. With human biofield treatment, percentage of CD45+CD44hiCD25− and percentage of CD8+ T cells returned to levels comparable to those seen in the popliteal lymph nodes of the PBS-injected mice (Figure 3). The graphs in Figure 3 illustrate the mean values of the eight mice in the three groups. A representative FACS plot of cells from the popliteal lymph nodes or spleen (mouse closest to the mean value) is displayed next to each graph for each treatment group. The displayed gate frequencies are based on total lymphocytes.

In the spleen, FACS analysis revealed no significant changes in lymphocytes with cancer (CA) versus PBS (PBS) compared to lymphocytes from the popliteal lymph nodes. However, the percentage of CD11b+ splenic macrophages was significantly elevated in mice with cancer (CA) compared to the percentage of CD11b+ macrophages in the PBS-injected mice (PBS). Human biofield treatment significantly decreased percentage of CD11b+ macrophages and returned their levels to those seen in the spleen of PBS-injected mice (PBS) (Figure 3).
Figure 2  Effect of human biofield on serum cytokine/chemokine levels
Human biofield treatment (TR) significantly decreased MIP-2, MIG, IL-1β, IL-2, and IFN-γ from their high levels in the mock treatment group (CA) to the levels found in the control group (PBS). Bars are means ± standard error of the means. n = 8 mice per group. MIP-2: macrophage inflammatory protein-2; MIG: monokine induced by interferon-gamma; IL: interleukin; IFN-γ: interferon-gamma; PBS: phosphate-buffered saline.
4 Discussion

4.1 Summary

To assess the effects of human biofield treatment an aggressive mouse breast cancer model was studied, and demonstrated large tumor formation and metastasis to the popliteal lymph nodes. This was the first time that immune function was studied in this breast cancer model, and that human biofield therapy was able to produce significant positive changes in immune function. Two separate experiments demonstrated significant changes in eight of the eleven serum cytokine/chemokine markers in tumor-laden mice. These findings suggest that these serum cytokine/chemokine markers have a role in this breast cancer model. Although no significant changes were recorded in tumor size and metastasis between the mock-treated group and the human biofield-treated group, human biofield treatment by a single practitioner produced significant decreases in five serum cytokine/chemokine markers that were up-regulated with cancer. Six other cytokines whose levels increased with cancer were not significantly affected by human biofield treatment. FACS analysis of subgroups of immune cells revealed significant changes in large number of lymphocyte types, B cells, CD8 T cells and macrophages (CD11b+) with cancer compared to the control. Human biofield therapy significantly decreased the percentage of CD4 CD44loCD25+ and percentage of CD8 T cells in

Figure 3 Flow cytometry scatter plots of a representative mouse in each box and graph from the popliteal lymph nodes of mice with cancer treated with human biofield (TR) compared to mice with cancer and mock treatments (CA), and mice that were PBS-injected without cancer (PBS)

With human biofield treatment (TR), percentages of CD8+ and CD44+CD25+ in the popliteal lymph nodes and CD11b+ macrophages in the spleen were increased with cancer (CA) and returned to levels comparable to those levels found in PBS-injected mice (PBS). Bars are means ± standard error of the means. n = 8 mice per group.
the popliteal lymph nodes, and the percentage of CD11b+ macrophages in the spleen, which were up-regulated with cancer, and brought down to levels comparable to the control mice without cancer (PBS). Thus human biofield therapy has significant effects on the immune system.

4.2 In cancer, macrophages are inhibited by human biofield treatment

Human biofield therapies appear to target specifically CD11b+ macrophages. This effect is further confirmed by our finding that the levels of macrophage-related cytokines, IL-1α, IL-1β, and MIP-2, are brought to normal levels with human biofield treatment. Macrophages have been shown to promote tumor progression and metastasis in breast cancer[46,47]. Macrophage suppression by propranolol in stressed mice injected with 66cl4 cells was shown to inhibit metastasis but not primary tumor growth, which corroborates our findings[48]. Future studies with macrophages and human biofield treatment may reveal how this therapy elicits effects on macrophages. Numerous studies with various types of cells and energy medicine modalities have shown effects on the growth and activity of cells such as bacterial[24,49], normal osteoblasts, tenocytes and fibroblasts[50], cancer cells[50], Jurkat T cells[19], while other studies found variable effects[25,51,52]. Interestingly Chinese herbal medicine was shown to particularly target macrophages in pancreatic cancer in mice[53].

4.3 Cytokines

Affected by human biofield treatment IL-1β and IFN-γ are secreted by activated T lymphocytes[54]. IFN-γ is a cytokine that promotes innate and adaptive immune responses, and has a role in tumor development[55]. Since both IL-1 and IFN-γ have been shown to have opposite effects at different stages of tumor development[56], it is difficult to know definitively if the decrease in IL-1 and IFN-γ with biofield treatment would inhibit cancer progression.

Two other cytokines, IL-2 and MIG, were significantly affected by the biofield practitioner. IL-2 is necessary for the growth and function of T cells and facilitates the production of immunoglobulins by B cells. MIG is a T-cell chemoattractant, which is induced by IFN-γ[57], and belongs to the same CXC chemokine family as MIP-2. Thus, the practitioner appears to have a significant effect on CXC chemokine members and IFN-γ suggesting that this pathway is particularly sensitive to human biofield effects. In general, the cytokine/chemokine data from the serum showed that human biofield therapy brought cytokine levels down from higher levels induced by cancer to normal levels.

Interestingly, the cytokines affected in this breast cancer model were entirely different from wound healing models, even though the cancer cells were injected into the foot of the mouse and may be hypothesized to parallel some aspects of wound healing. IL-6, MCP-1, and tumor necrosis factor α are elevated in wound healing models[57,58] but not in this study of breast cancer, except for IL-1β, which is elevated in both models.

4.4 Tumor growth

The role of the immune response in controlling solid tumors is controversial[59], but chronic stress modulators from the sympathetic nervous system and the neuroendocrine system have been shown to have a profound effect on immune cells and the severity of disease in breast and ovarian cancer[48,60]. Physiologic stressors are known to activate the sympathetic nervous system to release norepinephrine, which activates adrenergic receptors and the cAMP/protein kinase A signaling pathway, both of which have been found in breast cancer cells and immune cells, such as T lymphocytes and macrophages[48,61,62]. Interestingly chronic stress induced by a few hours of restraint, increased metastasis but not tumor growth in a mouse model of breast cancer[48]. Similar results were found in our mouse model in which TR treatment changed immune responses but not tumor size. In both experimental models, cancer cells were injected into mice and the tumor size was not affected, perhaps due to the different paths of primary tumor growth versus metastasized tumor growth. Another reason may be that in these mouse models of breast cancer, the primary tumors at the site of injection grow too fast and aggressively to allow for immune modulation of tumor size.

4.5 Lymphocytes

The present study demonstrated significant changes in the cells in the popliteal lymph nodes with cancer—percentages of CD4+CD44hiCD25+, CD44hiCD25−, CD44loCD25+, CD19+ (B cells), CD8+ T cells, and CD11b+ macrophages. Human biofield treatment from the single practitioner was only able to decrease significantly percentage of CD4+CD44loCD25+ and percentage of CD8+ T cells. CD25 is the alpha chain of the IL-2 receptor and is found on activated T and B cells. In this breast cancer model, IL-2 was elevated with cancer but was significantly reduced with treatment by the practitioner. CD44 and CD25 are expressed on activated T lymphocytes. CD44 is a multifunctional cell surface molecule involved in many cell activities and is found on cancer cells. CD8 is a transmembrane glycoprotein and is a co-receptor for the T cell receptor, and is found on the surface of cytotoxic T cells, on some natural killer cells, cortical thymocytes and some dendritic cells. Cytotoxic CD8+ T lymphocytes have a major role in tumor-specific cellular immunity by attacking tumor cells. In fact, better breast cancer patient survival was associated with total number of CD8+ cells along with other variables[63]. Our study has shown that CD8+ cells were significantly increased in the
draining popliteal lymph nodes with breast cancer in mice and that the practitioner had a significant effect of bringing these levels back to normal. How this would affect disease progression is not known.

4.6 Replication of human biofield studies

Another goal of this study was to determine if human biofield results could be replicated. In a previous study from our laboratory, several Therapeutic touch practitioners treating the same mouse model were able to significantly down-regulate IL-1α, IL-1β, MIP-2 and MIG[27]. In the present study these same cytokines were also decreased significantly by a well-known healer. These data demonstrate that human biofield effects on serum cytokines are reproducible. Analysis of lymphocyte populations in the popliteal lymph node and spleen produced different results from the previous work, except for splenic CD11b+ macrophages that were significantly decreased to control levels (PBS) in both studies. One difference between the two biofield studies was that Therapeutic touch practitioners treated the mice twice a week for a total of seven times, while the healer in this study treated mice only twice. Previous in vitro work in our laboratory has shown that there is a dose response to human biofield therapies[28]. With the additional treatment, metastasis was also significantly decreased in the previous study[27]. We don’t know if additional treatments in the present study would have eventually produced changes in metastasis levels.

Although the human biofield did not produce significant changes in tumor size or metastasis to the popliteal lymph nodes, significant changes were found in immune responses apparent in the down-regulation of specific lymphocytes and serum cytokine/chemokines. The human biofield appears to restore these cells and factors back to normal levels or homeostasis. In addition, these experiments produced novel reproducible changes in markers for breast cancer to be studied in the future. Since the immune system does play a role in cancer suppression and promotion by affecting the tumor microenvironment and by directly affecting tumor cell survival[29], the long-term outcome of this immune modulation is not known. The acute nature of this particular breast cancer model probably does not allow enough time for changes in the immune system to affect tumor size or metastasis, which was so rapid over the four-week study. A breast cancer model that is less severe should be tested to determine if these changes in immunity can affect disease outcome. Future macrophage studies involving human biofield therapy would be of value perhaps to elucidate a particular pathway affected by energy medicine.

5 Conclusion

These data in a mouse model of breast cancer demonstrate that human biofield therapy has a significant effect on immune responses, but not on tumor size or metastasis. Specifically, serum cytokine levels and specific lymphocyte populations and macrophages up-regulated by cancer, were significantly decreased and brought back to normal control levels with human biofield treatment.

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7 Competing interests

The authors declare that they have no competing interests.

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