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Effect of Electroacupuncture and Transcutaneous Electrical Acupoint Stimulation on Tumor Related T Cells

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ABSTRACT

Objective of this study was to determine the effect of electroacupuncture and transcutaneous electrical acupoint stimulation on tumor-related T cells. C57BL/6J mice used in the study were randomly divided into control and electrical stimulation group. The tumor model was established by subcutaneously injecting MC38 colorectal adenocarcinoma cells into mice. The mRNA expression of pro-inflammatory factors IL-10, IL-6 and 1L-1β of the mice was analyzed by RT-PCR. The numbers of CD8 + T cells and CD4 + T cells were analyzed by flow cytometry. The protein expression of PD-1 and CD39 of the mice was analyzed by Western blot. The metabolic rate of CD8 + T was analyzed by fluorescent nutrient stain. Results showed that the mRNA of IL-10, IL-6 and 1L-1β in the electric stimulation group decreased compared with that in the control group (P<0.05). On the 15th and 25th day, the tumor volume of the mice in the electric stimulation group decreased compared with that in the control group (P<0.05). Compared with the control group, the percentage of living cells of CD8+T and CD4+T in the electric stimulation group increased (P<0.05). The protein expression of PD-1 in the electric stimulation group was higher and that of CD39 in the electric stimulation group was lower than that in the control group (P<0.05). The rate of oxygen consumption and extracellular acidification of CD8 + T cells increased after electric in stimulation group compared with the control group (P<0.05). To conclude, that electroacupuncture and transcutaneous electrical acupoint stimulation could inhibit the occurrence of tumors, promote proliferation and activation of T cells, and strengthen the immune clearance of tumor cells.

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Authors' Contribution
YHC and SL collected the samples. YHC analysed the data. SL conducted the experiments and analysed the results. Both authors discussed the results and wrote the manuscript.

Key words
Electroacupuncture and transcutaneous electrical acupoint stimulation, Tumor; T cell, Cellular immunity

INTRODUCTION

Cancer is a recessive disease traditionally classified by cell and tissue origin. The immune system can recognize and reject cancer (Motomura *et al*., 2019). When tumors are produced from chronically inflamed tissues, tumor cells can also metastasize there. Chronic infiltration of leukocytes (such as myeloid cells activated by type 2 cytokines and immunosuppressive B, T and myeloid subtypes) destroys the targeted clearance of T cells, thus assisting tissue-based procedures, such as angiogenesis, lymphangiogenesis and matrix remodeling, and supporting tumor progression (Han *et al*., 2009). Regardless of the origin, a common feature of all cancers is the existence of various combinations of immune cells (Mahon *et al*., 2019). This kind of infiltration has various influences on cancer cells (Fornia *et al*., 2020). It is known that cancer cells will develop mechanisms to evade effective anti-tumor immunity, such as exonucleases CD39 and CD73. They act synergistically to convert extracellular immune stimulation ATP into adenosine (Tantawy *et al*., 2019). CD39 is expressed by different immune cell populations and cancer cells of different tumor types, and supports tumor to escape immune recognition and destruction (Nakano *et al*., 2020). Therefore, increasing extracellular ATP and simultaneously decreasing adenosine concentration in tumors can lead to effective anti-tumor immunity (Dineen *et al*., 2019). Local immunosuppression in tumor microenvironment is a sign of many cancers. The reactivation of T cell function by checkpoint blockade can lead to amazing clinical reaction, but it is only effective among a few patients (Hojman *et al*., 2019). Immunosuppressive pathways that play a role in tumors significantly affect the efficacy of immunotherapy. Acupuncture is a treatment technique used in traditional Chinese medicine. It involves penetrating the skin with fine sterile needles at well-defined acupoints which may be exciting muscle/skin nerve complex with high nerve endings density (Jiang *et al*., 2019). Needles are mainly stimulated manually or electrically. In manual

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acupuncture, the needle should be twisted back and forth. In addition to traditional manual acupuncture, new acupuncture methods, such as electroacupuncture and transcutaneous electrical acupoint stimulation, are becoming more and more popular (Tamura *et al*., 2019). In electroacupuncture, stimulation currents of various parameters are applied to acupoints through needles. At the same time, in transcutaneous electrical acupoint stimulation, electric pulses are transmitted to the skin of acupoints through electrodes (Huffman *et al*., 2019). It has been reported that electroacupuncture and transcutaneous electrical acupoint stimulation can activate the immune system of tumor mice and inhibit the occurrence of tumor to some extent (Chapman *et al*., 2020). The regulatory effects of electroacupuncture and transcutaneous electrical acupoint stimulation on tumor-related T cells have not been reported. In this study, we investigated the regulatory effects of electroacupuncture and transcutaneous electrical acupoint stimulation on tumor-related T cells.

MATERIALS AND METHODS

Experimental mice

C57BL/6J mice (obtained from Jackson Laboratory) were used in experimental research, and they were all raised without specific pathogens. During the experiment, mice were free to eat and drink water. The room temperature was set at ±22 2°C. After the experimental treatment, mice were euthanized and received tumor removal. The tumor dissociation kit was used to dissociate the tumor into single cells. All animal studies were conducted according to the protocol approved by the Ethics Committee of Merck Research Laboratory.

Mouse tumor model and electrical stimulation group treatment

C57BL/6J mice were injected subcutaneously with 500,000 syngeneic mouse MC38 colorectal adenocarcinoma cells suspended in phenol red-free DMEM (without additives), and the model was established for about 10 days. Once the average tumor volume reached 50-60μmm^3, the electrical stimulation group would begin to give treatment: mice in the group were kept in a fixed device without anesthesia. The design of this system is not only convenient for acupuncture research, but also makes experimental rats feel comfortable and relieve stress. A pair of stainless steel needles with a diameter of 0.25 mm were inserted into DU26 (depth of 5 mm) and DU16 (depth of 7.5 mm). In EA, continuous wave stimulation was performed at 2 Hz frequency and 0.2 mA intensity for 30 minutes. In TAES, the same transcutaneous instrument and continuous wave were applied to two acupoints for 30 minutes, with frequency of 2 Hz and current intensity of 1 mA. The electrical stimulation group received treatment twice a day until the end of the experiment.

Experimental grouping

According to the experimental requirements, all mice were randomly divided into the following two groups: control group (mice with tumor model established by subcutaneous injection of syngeneic mouse MC38 colorectal adenocarcinoma cells, n=15); electrical stimulation group (after the mouse tumor model was successfully established, the electrical stimulation group was given transcutaneous electrical acupoint stimulation, n=15).

Real-time quantitative PCR

Tumor tissue of mice was extracted, and RNA in a tumor tissue sample was used. cDNA was prepared by using large capacity cDNA reverse transcription kit (Thermo Fisher Scientific). According to the instructions of Fluidigm Biomark Manufacturer (Fluidigm), gene-specific preamplification was carried out. Two unlabeled primers (Table I) were used for real-time quantitative PCR of Fluidigm Biomark at a concentration of 900 nmol/L. Each primer was used together with 250 nmol/L FAM-labeled probe (Thermo Fisher Scientific), and TaqMan Universal PCR Master Mix was used together with UNG. Samples and primers were operated on the 96.96 array according to the manufacturer's instructions (Fluidigm). Ubiquitin levels were measured in separate reactions and normalized by the -ΔΔCt method.

Table I. Primers for RT-PCR.

Group	5'-3'
IL-10	F CCCAGACCCTACTCAGAT R TTGTCGAAGAGAACCTG
IL-6	F GCAGCTACCTTCTTGCCCGTG R GTCGTTGCTCTCTCCTTG
1L-1β	F AAGTTTCTCCCAAGATACA R AGGCAAATTGGTTATAT
GAPDH	F TCCCTCAAGGTCAGCAA R AGACACAGGATACATT

Detection of tumor volume

Tumor volume was measured twice a week. The tumor size was calculated as follows: tumor volume (mm^3)= major axis (mm)× minor axis (mm)× minor axis (mm)/2. Animal health care and experimental procedures were carried out in animal facilities approved by the Health Experimental Center of Japan Health Science Foundation. All the schemes had been approved by the agency's Animal

Care and Use Committee, and were carried out according to the animal experiment regulations of Eisai Co, Ltd.

T cell separation in vitro and flow cytometry analysis

Lymphocytes were isolated from the lymph nodes and spleen of naive C57BL/6J mice. Tissue was mechanically destroyed and passed through a 70-μm filter, and ACK lysis buffer (Gibco; cat# A1049201) was used to remove red blood cells. The negative selection kit (Miltenyi Biotec; Catalogue No. 130-104-075) was used to separate CD8+T cells and CD4+T cells. Cells were inoculated in 6-well tissue culture plates with plate-bound antibodies. Isolated T cells were stained in PBS for 30 minutes, washed and analyzed on LSRII or LSRFortessa flow cytometer (BD Biosciences). All flow antibodies were purchased from BD Biosciences as follows: CD8 (# 559250), CD4(#564108). All flow cytometry data were analyzed using Flow Jo (TreeStar software).

Western blot analysis

Mice were killed by deep anesthesia with 4% chloral hydrate, and cell lysis was carried out in M-PER buffer (Thermo Fisher Scientific; Catalog No.78501) with 0pierce protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific; catalogue No.88668). The protein expression of PD-1 and CD39 was studied by western blot analysis. Protein homogenate was prepared by rapid homogenization in 10 volumes of lysis buffer (2μmMEDTA, 10μmMEGTA, 0.4%NaF and 20μm MTris-HCl, pH 7.5). For each sample, the preparation concentration of protein lysate was 12μg/ lane, and it was fractionated on 12% SDS-polyacrylamide gel. The electro imprinted protein was transferred to nitrocellulose membrane (Abkim Biotechnology Company). The blot was compared with mouse monoclonal antibody anti-PD-1 (1:1000) and rabbit polyclonal antibody anti-CD39 (1:2000; Abkim Biotechnology Company). PD-1 and CD39 bands on immunoblotting were observed by enhanced chemiluminescence method (ECL kit, Santa Cruz Biotechnology Company). PD-1 and CD39 protein bands and β-actin bands were scanned by ChemiImager 5500 V2.03 software. IDV was calculated by Fluor Chen 2.0 software and normalization was carried out with by β-actin.

Absorption measurement of metabolic rate of T cells

All fluorescent nutrient stains were purchased from Sigma-Aldrich. About 250,000 activated CD8+T cells were placed in 400μLRPMI medium and the concentration of one marker was as follows: 2-NBDG (100μg/ mL; # N13195), BODIPY (1.25μg/mL; D3922), C12-BODIPY (1μmol/L;# D3822) and C16-BODIPY(0.5μmol/L;# D3821). Before washing and surface staining, cells were incubated at 37°C for 30 minutes for flow cytometry analysis.

Statistical analysis

The obtained data were expressed as mean±SD. GraphPad Prism 6.0 was used to analyze the data. (GraphPad Software Inc, Jolla, California, USA). According to chi-square or ANOVA, Tukeys test was performed. P <0.05 indicated statistically significant difference.

RESULTS

Table II shows that the effect of electric stimulation on mRNA expression of mouse pro-inflammatory factors (IL-10, IL-6 and 1L-1β). The mRNA expression of the cytokines is decreased after electrical stimulation (*P<0.05*) (Fig. 1). The tumor volume was also decreased on 15th and 25th day after electrical stimulation (*P<0.05*). The analysis of T lymphocytes by flow cytometry showed that the percentage of living cells of CD8+T and CD4+T in mice of two groups was increased after electrical stimulation (*P<0.05*), indicating that electroacupuncture and transcutaneous electrical acupoint stimulation could promote the activation of T lymphocytes.

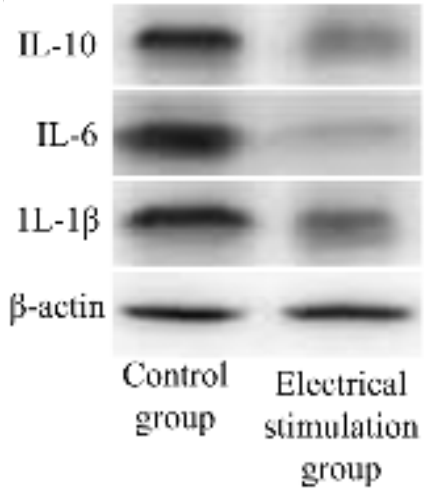

Fig. 1. RT-PCR analysis of pro-inflammatory factor mRNA expression.

Table III shows the effect of electrical stimulation on the protein expression of PD-1 and CD39. The PDI is down regulated, whereas CD39 is upregulated in electrical stimulation group compared with control group(*P<0.05*). (Fig. 2). In addition, the oxygen consumption rate and rate of extracellular acidification of mouse CD 8+T cells is also increased in the electrical stimulation group than in control group (*P<0.05*), indicating that the metabolism of mouse T cells and their proliferation and activation was promoted in electrical stimulation group.

Table II. Effect of electrical stimulation on mRNA expression (±s) of pro-inflammatory of cytokines mouse tumor volume and percentage of living cells analyzed by RT-PCR.

		Control group	Electrical stimulation group	t value	P value
IL-10		2.16±0.24	1.38±0.12	5.239	0.026
IL-6		2.46±0.28	1.24±0.18	6.175	0.014
1L-1β		2.33±0.25	1.09±0.11	5.435	0.035
Mouse tumor volume measurement	15d (mm^3)	637.25±24.08	418.76±15.42	5.638	0.034
	25d (mm^3)	968.45±38.26	529.15±23.44	4.109	0.015
Flow cytometry analysis	CD8 + T (%)	15.37±4.16	26.52±6.29	6.382	0.015
	CD4 + T (%)	19.75±5.02	30.58±7.18	5.074	0.037

Table III. Effect of electrical stimulation on the protein expression of PD-1 and CD39 and metabolism of CD8+T cells by western blot analysis.

Group		Control group	Electrical stimulation group	t	P
PD-1		1.26±0.11	2.34±0.26	4.608	0.022
CD39		2.14±0.24	1.15±0.12	6.371	0.031
Metabolism analysis of cd8+t cells	Oxygen consumption rate (pmol/min)	18.35±2.15	37.24±4.19	5.316	0.268
	Extracellular acidification rate (mph/min)	13.71±2.33	25.16±3.08	4.721	0.017

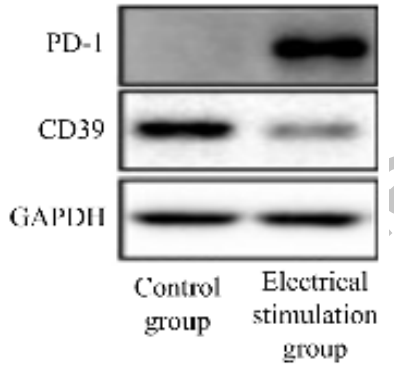

Fig. 2. Western blot analysis.

DISCUSSION

Electroacupuncture and transcutaneous acupoint stimulation are modern therapies derived from traditional acupuncture, which have attracted increasing attention in recent years (La *et al*., 2019). Acupuncture as an ancient non-drug therapy originated in China has been widely used all over the world in recent years. Recently, more and more studies have shown that acupuncture can effectively regulate the function of the immune system (Wei *et al*., 2020). In this study, the mice with tumor were used as the research objects, and the regulatory effects of electroacupuncture and transcutaneous acupoint stimulation on T cells were investigated. It was found that electroacupuncture and transcutaneous acupoint effectively inhibited the occurrence of tumor cells and reduced the tumor size.

According to the concept of nutrition competition in TME between effector T cells and cancer cells, enhancing the ability of T cells to obtain nutrition and utilizing the "adaptability" of nutrition can achieve better tumor clearance rate (Siemens *et al*., 2020). Therefore, the cell metabolism of CD8+T cells was tested in this study. The oxygen consumption rate and rate of extracellular acidification were increased after electroacupuncture and transcutaneous electrical acupoint stimulation. In this study, electrical stimulation up-regulated oxygen consumption rate and rate of extracellular acidification in CD8+T cells in vivo. Tumor cells outperform T cells in nutrition competition, which is one of the mechanisms of tumor immunosuppression. Lee *et al*. (2019) found that enhancing the metabolism of tumor cells can transform murine tumor regression lines into progressive cancers (Lee *et al*., 2019). Under the condition of nutrition competition, it may be beneficial to promote T cell metabolism with electrical stimulation. Immune cell metabolism is increasingly appreciated because of its influence on immune cell function. Here, we clarified some metabolic effects of electrical stimulation group on T lymphocytes in tumor, so as to better understand the mechanism of tumor clearance. At present, combined tumor treatment schemes are attracting increasing attention. Understanding the mechanism of increased metabolism by electroacupuncture and transcutaneous electrical acupoint stimulation can

save the environment of T cell proliferation and effector function. It is necessary to make people better understand the role of small molecules combined with immunotherapy in regulating immune cell metabolism. These insights may lead to enhanced treatment strategies, thus improving the prognosis of patients.

To further enhance the efficacy of cancer immunotherapy, the strategy of targeting tumor immune escape and/or resistance mechanism is of clinical significance. More and more evidences show that extracellular adenosine produced by extracellular nucleotide CD39 is the key metabolite that negatively regulates anti-tumor immunity (Stavrakis *et al*., 2020). As we all know, Treg promotes tumor progression by inhibiting anti-tumor immunity. It is known that many mouse tumor models can be infiltrated by Treg, and their depletion usually improves the anti-tumor immune response. In addition, the increased number of Treg is associated with poor prognosis of various types of human cancers (Chen and Zhong, 2013). Treg in tumor-bearing hosts is known to express CD39. Immune escape of cancer cells is the main mechanism of cancer malignant tumors, which is mainly due to the failure of CD8+T cells to recognize tumor antigens. PD-1 signaling pathway is the key regulator of CD8+T cell failure (Voloshin *et al*., 2020). Our study has proved that the CD39 decreased in mice after electrical stimulation. The improvement of Treg density by tumor infiltrating CD8+T cells indicated the re-enhanced anti-tumor immunity, and the induction treatment effect could be enhanced by anti-PD-1 antibody. According to the research results, the protein expression of PD-1 in electrical stimulation group was higher than that in control group, indicating that electrical stimulation inhibited the occurrence of immunosuppression and enhanced the tumor immune effect of T cells. Secondly, the decrease of CD39 expression led to the decrease of ATP degradation in tumor microenvironment and caused an increase in the proliferation of CD8+effector T cells, thus potentially improving the anti-tumor T cell response.

Anti-tumor immune response usually depends on treatment time. Recent findings show that about one week of analysis is enough to reveal the changes of immune cell population. In our study, flow cytometry analysis also showed that the electrical stimulation induced the activation of CD8+T and CD4+T cells after treatment. As a physiological countermeasure to improve the harmful overactivation of innate immunity, compensatory anti-inflammatory reactions usually occur, including secretion of anti-inflammatory cytokines by monocytes and T cells (such as IL-10 and IL-6), down-regulation of inflammatory cell surface receptors on neutrophils and weakening of monocyte response to bacterial endotoxin, which is known to cause transient immunosuppression (Keilani *et al*., 2019). Usually, this immunosuppression is temporary and usually return to normal. However, when it is serious and persistent in some cases, this immune imbalance is considered as the cause of postoperative infection complications. Long-lasting immunosuppressive reaction is still a clinical challenge. Our research found that electrical stimulation balanced the inflammatory reaction caused by tumor reaction and reduced the mRNA expression of IL-10, IL-6 and 1L-1β.

To sum up, electroacupuncture and transcutaneous electrical acupoint stimulation has been proved to inhibit the occurrence of tumor cells and promote the proliferation and activation of T cells, thus enhancing the immune clearance of tumor cells.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Chapman, E.J., Edwards, Z., Boland, J.W., Maddocks, M., Fettes, L., Malia, C., Mulvey, M.R., Bennett, M.I., 2020. Practice review: Evidence-based and effective management of pain in patients with advanced cancer. *Palliat. Med*., **34**: 444-453. https://doi.org/10.1177/0269216319896955

Chen, M.J. and Zhong, J.C., 2013. Logotherapy method for creating and treatment of patients with advanced cancer and its preliminary evaluation. *Med. Philos*., **34**: 69-72.

Dineen, J., Maus, D.C., Muzyka, I., See, R.B., Cahill, D.P., Carter, B.S., Curry, W.T., Jones, P.S., Nahed, B.V., Peterfreund, R.A. and Simon, M.V., 2019. Factors that modify the risk of intraoperative seizures triggered by electrical stimulation during supratentorial functional mapping. *Clin. Neurophysiol*., **130**: 1058-1065. https://doi.org/10.1016/j.clinph.2019.03.006

Fornia, L., Rossi, M. and Rabuffetti, M., 2020. Direct electrical stimulation of premotor areas: different effects on hand muscle activity during object manipulation. *Cereb Cortex*, **30**: 391-405. https://doi.org/10.1093/cercor/bhz139

Han, J., Ran, X.Z. and Ai, G.P., 2009. Norepinephrine improves bone marrow mesenchymal stem cells proliferation through AR/PKC-dependent signaling. *J. Third Milit. Med. Univ*., **31**: 2295-2298.

Hojman, P., Brolin, C. and Nørgaard-Christensen, N., 2019. IL-6 release from muscles during exercise is stimulated by lactate-dependent protease activity.

Am. J. Physiol. Endocrinol. Metab., **316**: 940-947. https://doi.org/10.1152/ajpendo.00414.2018

Huffman, W.J., Subramaniyan, S. and Rodriguiz, R.M., 2019. Modulation of neuroinflammation and memory dysfunction using percutaneous vagus nerve stimulation in mice. *Brain Stimul*., **12**: 19-29. https://doi.org/10.1016/j.brs.2018.10.005

Jiang, X., Tian, Y. and Xu, L., 2019. Inhibition of triple-negative breast cancer tumor growth by electroacupuncture with encircled needling and its mechanisms in a mice xenograft model. *Int. J. med. Sci*., **16**: 1642-1651. https://doi.org/10.7150/ijms.38521

Keilani, M., Kainberger, F., Pataraia, A., Hasenhrl T. and Crevenna R., 2019. Typical aspects in the rehabilitation of cancer patients suffering from metastatic bone disease or multiple myeloma. *Wien Klin. Wochenschr*., **131**: 567-575. https://doi.org/10.1007/s00508-019-1524-3

La, G., Zhou, M., Lim, J.Y., Oh, S. and Zhong, L., 2019. Proteomics and transcriptomics analysis reveals clues into the mechanism of the beneficial effect of electrical stimulation on rat denervated gastrocnemius muscle. *Cell Physiol. Biochem*., **52**: 769-786. https://doi.org/10.33594/000000054

Lee, J.E., Anderson, C.M., Perkhounkova, Y., Sleeuwenhoek, B.M. and Louison, R.R., 2019. Transcutaneous electrical nerve stimulation reduces resting pain in head and neck cancer patients: a randomized and placebo-controlled double-blind pilot study. *Cancer Nurs*., **42**: 218-228. https://doi.org/10.1097/NCC.0000000000000594

Mahon, B.Z., Miozzo, M. and Pilcher, W.H., 2019. Direct electrical stimulation mapping of cognitive functions in the human brain. *Cogn. Neuropsychol*., **36**: 97-102. https://doi.org/10.1080/02643294.2019.1630375

Motomura, K., Terasawa, Y. and Natsume, A., 2019. Anterior insular cortex stimulation and its effects on emotion recognition. *Brain Struct. Funct*., **224**: 2167-2181. https://doi.org/10.1007/s00429-019-01895-9

Nakano, J., Ishii, K. and Fukushima T., 2020. Effects of transcutaneous electrical nerve stimulation on physical symptoms in advanced cancer patients receiving palliative care. *Int. J. Rehabil. Res*., **43**: 62-68. https://doi.org/10.1097/MRR.0000000000000386

Siemens, W., Boehlke, C., Bennett, M.I., Offner, K., Becker, G. and Gaertner, J., 2020.Transcutaneous electrical nerve stimulation for advanced cancer pain inpatients in specialist palliative care-a blinded, randomized, sham-controlled pilot cross-over trial. *Support Care Cancer*, **28**: 5323-5333. https://doi.org/10.1007/s00520-020-05370-8

Stavrakis, S., Stoner, J.A., Humphrey, M.B., Morris, L., Filiberti, A., Reynolds, J.C., Elkholey, K., Javed, I., Twidale, N., Riha, P., Varahan, S., Scherlag, B.J., Jackman, W.M., Dasari, T.W. and Po, S.S., 2020. TREAT AF (transcutaneous electrical vagus nerve stimulation to suppress atrial fibrillation): A randomized clinical tial. *ACC Clin. Electrophysiol*., **6**: 282-291. https://doi.org/10.1016/j.jacep.2019.11.008

Tamura, M., Sato, I. and Maruyama, T., 2019. Integrated datasets of normalized brain with functional localization using intra-operative electrical stimulation. *Int. J. Comput. Assist. Radiol. Surg*., **14**: 2109-2122. https://doi.org/10.1007/s11548-019-01957-7

Tantawy, S.A., Elgohary, H.M.I. and Abdelbasset, W.K., 2019. Effect of 4 weeks of whole-body vibration training in treating stress urinary incontinence after prostate cancer surgery: A randomised controlled trial. *Physiotherapy*, **105**: 338-345. https://doi.org/10.1016/j.physio.2018.07.013

Voloshin, T., Kaynan, N., Davidi, S., Porat, Y., Shteingauz, A., Schneiderman, R.S., Zeevi, E., Munster, M., Blat, R., Brami, C.T., Cahal, S., Itzhaki, A., Giladi, M., Kirson, E.D., Weinberg, U., Kinzel, A. and Palti, Y., 2020. Tumor-treating fields (TT Fields) induce immunogenic cell death resulting in enhanced antitumor efficacy when combined with anti-PD-1 therapy. *Cancer Immunol. Immunother*., **69**: 1191-1204. https://doi.org/10.1007/s00262-020-02534-7

Wei, W., Bai, W., Yang, Y., Li, Y., Teng, X.F., Wan, Y.X. and Zhu, J.C., 2020. Pulmonary protection of transcutaneous electrical acupoint stimulation in gynecological laparoscopic surgery: A randomized controlled trial. *Exp. Ther. Med*., **19**: 511-518. https://doi.org/10.3892/etm.2019.8245