Effects of transcutaneous acupoint electrical stimulation on the imbalance of Th_1, Th_2, Th_{17} and T_{reg} cells following thoracotomy of patients with lung cancer

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Abstract. An imbalance in the various T lymphocytes, including T-helper (Th)$_1$, Th_2 and Th_{17} cells, and regulatory T (T_{reg}) cells, has been associated with immune dysfunction, and may occur following thoracotomy of patients with lung cancer. The use of transcutaneous acupoint electrical stimulation (TAES) has previously been demonstrated to exert immunoregulatory effects; therefore, the present study aimed to evaluate whether TAES was able to attenuate postoperative immune suppression in patients with lung cancer. Thoracic surgical patients with lung cancer (n=27) underwent TAES (frequency, 2/100 Hz; intensity, 4-12 mA) at the bilateral large intestine 4, pericardium 6, small intestine 3 and San Jiao 6 acupuncture points for 30 min, prior to incision, and at 20, 44, 68, 92 and 116 h following thoracotomy. The number of Th_1, Th_2, Th_{17} and T_{reg} cells, and the protein and mRNA expression levels of related cytokines were measured by flow cytometry, ELISA and polymerase chain reaction, respectively. The balance of Th_1, Th_2, Th_{17} and T_{reg} cells in the peripheral blood of patients with lung cancer was disrupted following thoracotomy. TAES administration increased the percentage of Th_1 and Th_{17} cells, the protein expression levels of interleukin (IL)-2 and interferon-γ, the mRNA expression levels of T-bet and RAR-related orphan receptor-γt, and decreased the percentage of Th_2 cells, IL-10 protein expression levels, and GATA binding protein 3 mRNA expression levels. The results of the present study demonstrated that TAES was able to partially attenuate the postoperative immune depression of patients with lung cancer, by regulating the balance of Th_1, Th_2, Th_{17} and T_{reg} cells, and the expression levels of related cytokines and transcription factors; therefore, TAES may be considered to be a promising strategy for treating postoperative immune dysfunction in patients with lung cancer.

Introduction

Lung cancer is associated with the highest mortality rate (18.2%) of all types of cancer (1), and intervention typically involves surgical removal of the tumor, with a concomitant lymphadenectomy. Patients with lung cancer exhibit numerous immune abnormalities, including cellular immune dysfunction, cytokine alterations, microcirculatory disturbance and antigen presentation defects (2,3), which are exacerbated as a result of surgical trauma and postoperative pain. Furthermore, the immune dysfunction associated with surgical trauma may predispose patients to septic complications, multiple organ dysfunction, tumor spread or metastases, and mortality (4,5); therefore, it is important to develop strategies that are able to attenuate perioperative immune dysfunction in patients with lung cancer.

The activation and differentiation of T lymphocytes is required for anti-infection and anti-tumor immune responses (6). In addition, an imbalance in the relative levels of the various T-helper (Th) cells, including Th_1, Th_2 and Th_{17} cells, and regulatory T (T_{reg}) cells, has been associated with immunological disturbances (7). In a previous study, Th_{17} cells exhibited potent pro-inflammatory properties via the secretion of interleukin (IL)-17, IL-21 and IL-23. Furthermore, immunosuppressive effects of T_{reg} cells were detected, and were associated with an immunocompromised state (8). The identification of Th_{17} and T_{reg} cells progressed understanding of the mechanisms underlying immune dysfunction. An imbalance of Th_{17}/T_{reg} cells has been widely detected in human cancer, inflammatory and autoimmune diseases (9); however,

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Abbreviations: TAES, transcutaneous acupoint electrical stimulation; Th cells, T-helper cells; T_{reg} cells, regulatory T-cells; VAS, visual analogue scale

Key words: transcutaneous acupoint electrical stimulation, lung cancer, immunity, T-helper, regulatory T cells

to the best of our knowledge, it has yet to be associated with thoracotomy in patients with lung cancer.

Transcutaneous acupoint electrical stimulation (TAES) is a novel analgesic therapy used in the practice of physiotherapy in order to relieve pain associated with acute and chronic inflammatory conditions (10). Furthermore, TAES may serve as a relatively safe and noninvasive alternative to acupuncture, while providing comparable analgesic effects (11). According to the theory of traditional Chinese medicine, surgical trauma disrupts the balanced state of the human body, and disturbs the movement of Qi (vital energy) and blood (12). It has previously been suggested that the stimulation of acupoints may restore the balance of Qi, and facilitate recovery from bodily injury via effects on the central, autonomic nervous, immune, metabolic and endocrine systems (13,14). A previous study suggested that acupuncture may be used alongside existing therapies for the treatment of cancer and associated symptoms, due to its 'immune-boosting effects' (15). However, to the best of our knowledge, the effects of TAES on the immune state of patients with lung cancer following thoracotomy, and the underlying immunomodulatory mechanisms, are yet to be evaluated.

The present study aimed to evaluate the effects of TAES on the balance of Th_1, Th_2, Th_{17} and T_{reg} cells, and the expression levels of associated cytokines and transcription factors, following thoracotomy of patients with lung cancer.

Materials and methods

Participants and anesthesia. Between May 2012 and May 2013, 311 patients were diagnosed with lung cancer at the Cancer Hospital of Harbin Medical University (Harbin, China). A total of 113 patients met the inclusion criteria of the present study; however, 23 patients refused participation. Thus, a total of 90 patients (age, 18-65 years), who accepted thoracotomy for the treatment of American Society of Anesthesiology grade I-III (16), TNM stage I (T1-2N0M0) (17) lung cancer, were evaluated in the current randomized, controlled trial. Eligible patients had to meet the following criteria: i) Histologically confirmed lung cancer; ii) pathologic stage I; iii) no major organ (liver, kidney or heart) dysfunction; iv) no preoperative anticancer treatment; and v) no other cancer site besides the lungs. Diagnosis of patients with lung cancer occurred using histocytological methods, biopsies were obtained using a bronchofiberscope or thoracoscope. The exclusion criteria were as follows: i) The detection of a local or systemic infection; ii) a history of chronic pain or regular opioid consumption; iii) a body mass index of >40 kg/m^2; iv) evidence of body temperature disturbances; or v) they had previously experienced acupuncture/TAES therapies. The present study was approved by the Ethics Committee of Harbin Medical University, and written informed consent was obtained from all patients.

Anesthetization and thoracotomy of patients. Patients were anesthetized using midazolam (0.05 mg/kg; Jiangsu Nhwa Pharmaceutical Co., Ltd., Jiangsu, China), fentanyl (0.03 mg/kg; Yichang Humanwell Pharmaceutical Co., Ltd., Hubei, China) and propofol (2.5-3.0 mg/kg; AstraZeneca, Macclesfield, UK). Injection of patients with vecuronium bromide (0.08 mg/kg; Zhejiang Xianju Pharmaceutical Co., Ltd., Zhejiang, China) facilitated tracheal intubation using a double-lumen tracheal tube (Portex; Smiths Medical, Dublin, OH, USA). The lungs were ventilated mechanically (tidal volume, 8 ml/kg; ventilator frequency, 12-14 bpm; Aestiva 5 7100; 7GE Healthcare Life Support Solutions, Madison, WI, USA). Anesthesia was maintained using propofol (4-6 mg/kg/h) and remifentanil (Yichang Humanwell Pharmaceutical Co., Ltd.). The treatment of patients with remifentanil was initiated at a rate of 0.1 μg/kg/min, followed by 0.05 μg/kg/min increments or decrements, adjusted according to hemodynamic variables. If a mean arterial pressure of <60 mmHg or a heart rate of <45 beats/min were detected for >5 min, patients were treated with 10 mg ephedrine or 0.5 mg atropine. Ringer's acetate (Hunan Kelun Pharmaceutical Co., Ltd., Hunan, China) was administered at a rate of 6-8 ml/kg/h, in order to maintain basal fluid requirements. All of the patients underwent thoracotomy (18) with a rib spreader and wound retractor (tumed-Surgical Instrument & Hospital Supplies GmbH, Tuttlingen, Germany), and without rib excision. None of the patients required a perioperative blood transfusion, and all of the patients were operated on by the same surgical team, using a consistent operative procedure.

TAES procedure. An anesthesiologist performed TAES at the bilateral large intestine (LI) 4, pericardium (PC) 6, small intestine (SI) 3 and San Jiao (SJ) 6 acupuncture points. LI 4 is located on the dorsum surface of the hand, between the first and second metacarpal bones; PC 6 on the palmar aspect of the forearm, two 'cuns' above the transverse crease of the wrist, between the flexor carpi radialis and palmaris longus tendons; SI 3 on the ulnar side of the fifth digit, behind the head of the fifth metacarpal bone, the starting point of the little finger abductor muscle outer edge; and SJ 6 on the dorsal aspect of the forearm, two 'cuns' above wrist horizontal stripes between the ulna and radius. One 'cun' corresponds to the distance between the interphalangeal creases of the subject's middle finger. These acupoints were selected on the basis of the following findings from previous studies: Acupuncture at LI 4 and PC 6 was able to relieve abdominal pain, whereas it was associated with enhanced disease resistance and immunomodulation when performed at SI 3, and a reduction in incision pain associated with a rib wound when performed at SJ 6 (19-21).

The patients arrived at the laboratory at 9:00 AM on the assessment day, after which the study design and techniques were explained. The acupoints of all of the patients were swabbed with alcohol to reduce skin impedance, and were then covered with cutaneous self-adhesive electrode pads (size, 16 cm^2) connected to a HANS-200 device (Han's Acupoint Nerve Stimulator; Neuroscience Research Institute, Peking University, China). The acupoints were stimulated in the standard dense-and-disperse mode for 30 min, involving alternate stimulation at 2 Hz and 100 Hz every 3 sec (frequency, 2/100 Hz). The intensity of stimulation was set at 4-12 mA to initiate minor muscle contractions. Patients were informed that they may or may not feel the current.

The subjects were randomized equally into i) thoracotomy; ii) thoracotomy and sham TAES; and iii) thoracotomy and

TAES groups, using a computer-generated random list with coded sealed envelopes. Group 3 received TAES at the bilateral LI 4, PC 6, SI 3 and SJ 6 points for 30 min prior to incision, and at 20, 44, 68, 92 and 116 h following surgery. Group 2 received identical electrical stimulation to group 3; however, TAES was performed at sham points, which are located 4.0 cm obliquely superior and lateral to the LI 4, PC 6, SI 3 or SJ 6 acupoints, and are not in the meridian.

Sample collection and measurements. Blood samples were collected prior to surgery (basal), and at 24, 72 and 120 h post-operatively. Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood using density gradient centrifugation at 400 x g for 10 min at 4˚C and were analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA, USA). Plasma was stored at -70˚C for subsequent measurement of the expression levels of cytokines and transcription factors.

Determination of T-cell subsets. To determine the percentages of the various T-cell subsets, PBMCs were suspended at a density of $2x10^6$ cells/ml in phosphate-buffered saline. Cells were then incubated with mouse anti-human CD4 (11-0047-42; 1:1,000 dilution), IFN-γ (12-7319-42; 1:1,000 dilution), IL-4 (12-7049-42; 1:1,000 dilution), IL-17 (12-7178-42; 1:500 dilution), and forkhead box P3 (FoxP3; 12-4777-42; 1:500 dilution) monoclonal antibodies or isotype-matched immunoglobulin G controls (12-4998-82; 1:1,000 dilution) for 30 min at 37˚C. All antibodies were purchased from eBioscience, Inc. (San Diego, CA, USA). For intracellular IL-17A staining, the cells were treated with PMA (Sigma-Aldrich, St. Louis, MO, USA) at 50 ng/ml and ionomycin (Sigma-Aldrich) at 1 μM in the presence of GolgiStop (BD Pharmingen, San Diego, CA, USA) for 4 h. For the FoxP3 analysis, the cells were not stimulated. The stained cells were analyzed using a FACScan Cytometer equipped with CellQuest 6.0 software (BD FACSAria; BD Biosciences).

ELISA. The protein expression levels of IL-2, IFN-γ, IL-4, IL-10, IL-17 and transforming growth factor (TGF)-β, were measured using an ELISA (eBioscience, Inc.). All of the samples were analyzed in duplicate. The minimum detectable cytokine concentration was 10 pg/ml for IL-2, 20 pg/ml for IL-4, 2 pg/ml for IFN-γ, IL-10 and IL-17, and 60 pg/ml for TGF-β.

Reverse transcription-quantitative polymerase chain reaction. Total RNA was extracted from the PBMCs using the TRIzol® extraction kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Total RNA (2 μg) from each sample was reverse transcribed to cDNA in a 40 μl reaction mixture which contained 8 μl 5X reverse transcriptase moloney murine leukemia virus (M-MLV) buffer, 1 μl DTT, 2 μl dNTP mixture, 2 μl random primers, 1 μl reverse transcriptase M-MLV, 1 μl RNAsafe all Takara Biotechnology Co., Ltd., Dalian, China) and 23 μl RNase free ddH_2O. TaqMan primers and probes targeting human T-bet, GATA binding protein 3 (GATA3), RAR-related orphan receptor (ROR)-γt, and FoxP3 were used. Samples (2 μl cDNA) were analyzed using PrimeScript™ RT-PCR kit (Takara Biotechnology Co., Ltd.) and the ABI Prism 7900 Sequence Detection System (Applied Biosystems Life Technologies, Foster City, CA, USA). The PCR cycling conditions were as follows: 94˚C for 3 min, followed by 94˚C for 15 sec, 55˚C for 30 sec and 72˚C for 40 sec for 40 cycles, and final extension at 72˚C for 10 min. The primer pairs used were as follows: Forward, 5'-GCTGGAGAAAAGAAGACA AGAAAG-3' and reverse, 5'-AAGAAAAAACACACACCC ACACAC-3' for T-bet (496 bp); forward, 5'-AGGGAGTGT GTGAACTGTGGG-3' and reverse, 5'-CTTCGCTTGGGC TTAATGAGG-3' for GATA3 (253 bp); forward, 5'-GCA ATGGAAGTGGTGCTGGTT-3' and reverse, 5'-AGGATG CTTTGGCGATGAGTC-3' for RORγt (192 bp); and forward, 5'-CACGCATGTTTGCCTCTTCAGA-3' and reverse, 5'-GTA GGGTTGGAACACCTGCTGGG-3' for FoxP3 (235 bp). The mRNA expression levels of the target genes were normalized against GAPDH, and relative mRNA expression levels for each cytokine were calculated. Relative fold changes of gene expression were calculated using the ΔΔCq method using ABI Prism® 7900HT software, and the values were expressed as $2^{-\Delta\Delta Cq}$ (22), relative fold changes of target gene expression were normalized against GAPDH.

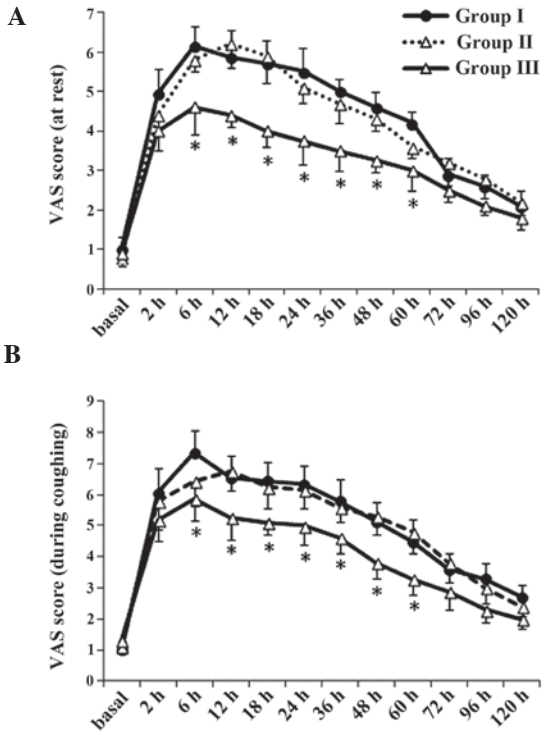

Figure 1. Postoperative pain (A) at rest and (B) during coughing assessed using a VAS prior to surgery (basal), and at 2, 6, 12, 18, 24, 36, 48, 60, 72, 96 and 120 h postoperatively. Data are presented as the mean ± standard deviation (n=27/group). *P<0.05 vs. group 1. VAS, visual analogue scale.

Pain measurement. Postoperative pain at rest and whilst coughing were assessed using a visual analogue scale (VAS), between 0 (pain free) and 10 (worst possible pain), prior to surgery (basal), and at 2, 6, 12, 18, 24, 36, 48, 60, 72, 96 and 120 h postoperatively. Intravenous treatment with 100 mg tramadol (Grünenthal GmbH, Aachen, Germany) was used for postoperative pain rescue, as required. Analgesic requirements, adverse events and durations of hospitalization, were also recorded.

Table I. Demographic and surgical information (n=27).

Characteristic	Group 1	Group 2	Group 3
Age (year)[a]	52.5±24.5	57.5±28.3	55.5±22.6
Gender (M/F)[b]	14/13	11/16	12/15
Weight (kg)[a]	64.8±10.9	62.8±10.2	67.5±8.5
Height (cm)[a]	172.5±6.8	176.5±8.2	171.5±5.5
TNM stage (I/IIa/IIb)[b]	7/11/9	6/13/8	6/10/11
Procedure[b]			
Lobectomy	16	13	14
Pneumonectomy	6	5	4
Bi-lobectomy	3	5	6
Wedge resection	2	4	3
Side (R/L)[b]	14/13	16/11	12/15
Thoracotomy length (cm)[a]	13.2±2.2	11.2±1.7	12.4±2.2
Blood loss (ml)[a]	157±62	145±65	167±72
Fluids (ml)[a]	1380±350	1220±430	1158±270
Lymph nodes resected[a]	13.5±2.5	14.5±3.5	16.0±4.5
Duration of surgery (min)[a]	160.5±34.5	151.5±27.5	145.5±32.5
Histology[b]			
Adenocarcinoma (n)	16	14	18
Squamous carcinoma (n)	9	9	6
Others (n)	2	4	3

Values are presented as [a]mean ± standard deviation or [b]n. M, male; F, female; TNM, TNM classification of malignant tumors; R, right; L, left.

Table II. Postoperative complications.

Complication	Group 1	Group 2	Group 3
Nausea/vomiting	9 (33.3)	8 (29.6)	3 (11.1)[a]
Pruritis	2 (7.4)	2 (7.4)	2 (7.4)
Hypotension	5 (18.5)	4 (14.8)	4 (14.8)
Respiratory depression	1 (3.7)	0 (0.0)	0 (0.0)
Desaturation	0 (0.0)	1 (3.7)	1 (3.7)
Pneumonitis	3 (11.1)	1 (3.7)	1 (3.7)
Atelectasis	1 (3.7)	0 (0.0)	1 (3.7)
Pulmonary embolism	0 (0.0)	1 (3.7)	0 (0.0)
Empyema	1 (3.7)	0 (0.0)	0 (0.0)
Infection	6 (22.2)	4 (14.8)	1 (3.7)[a]
Mortality	0 (0.0)	0 (0.0)	0 (0.0)

Values are presented as n (%). [a]P<0.05 vs. group 1.

Analysis

Data analysis. Power analysis was based on the results of our preliminary experiments comparing IL-2 protein expression levels 72 h after surgery among three groups, and yielded a sample size of n=21 (α=0.05; 1-β=0.9) for each group. Therefore, a sample size of n=30/group was used in the present study.

Statistical analysis. The normality of quantitative variables was analyzed by the Kolmogorov-Smirnov test. VAS scales were analyzed by repeated-measures analysis of variance (ANOVA) for inter-group comparison. Categorical data were compared using the χ^2 test or Fisher's exact test. The remaining data were analyzed using ANOVA or the Mann-Whitney U test. Statistical analyses were performed using the SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

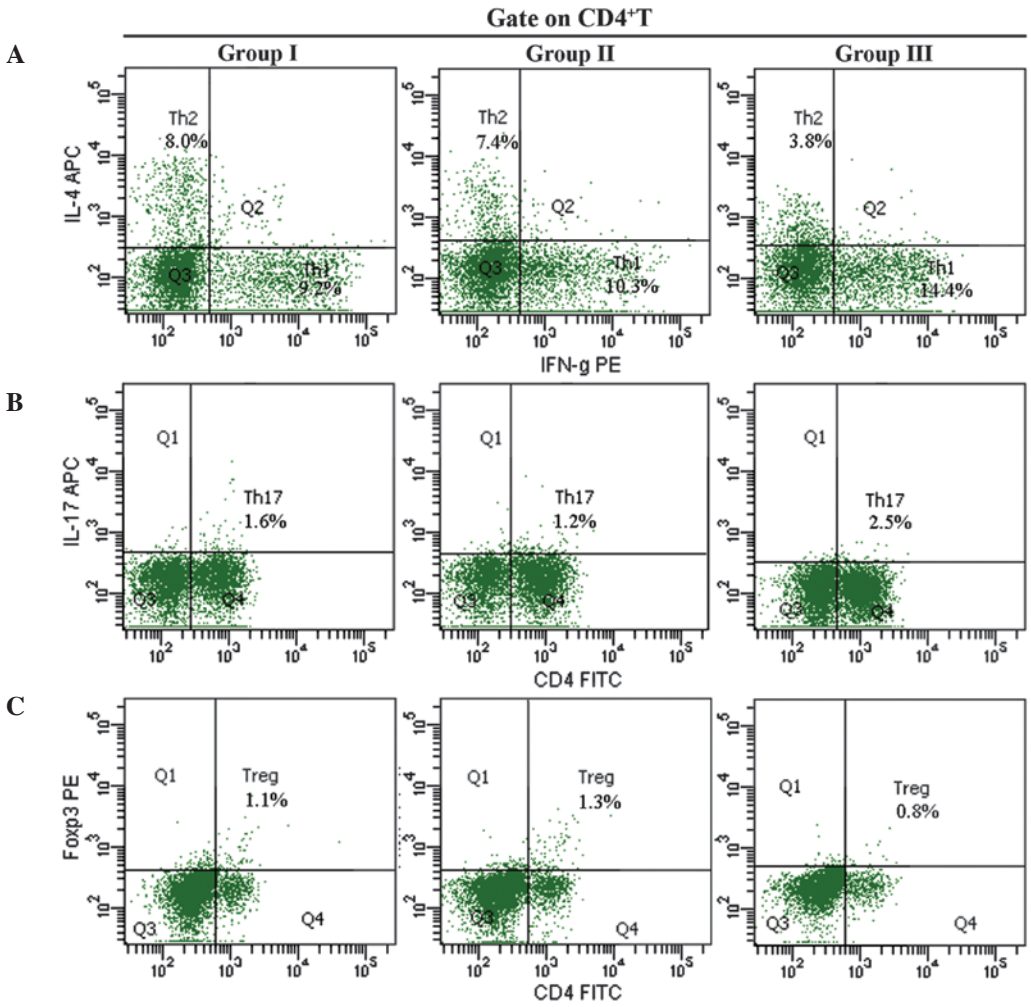

Figure 2. Percentages of (A) Th_1 and Th_2, (B) Th_{17} and (C) T_{reg} detected using fluorescence-activated cell sorting. Blood samples were collected at 72 h postoperatively, and peripheral blood mononuclear cells were suspended at a density of $2x10^6$ cells/ml. Representative binding patterns of intracellular monoclonal antibodies against IFN-γ, IL-4, IL-17 and FoxP3 in the various $CD4^+$ T-cell subsets.*P<0.05 vs. group 1. CD4, cluster of differentiation 4; IL, interleukin; APC, allophycocyanin; Th, T-helper cells; IFN, interferon, PE, phycoerythrin; FoxP3, forkhead box P3; T_{reg}, regulatory T-cells; FITC, fluorescein isothiocyanate.

Results

Demographic and surgical information. Of the 90 patients, nine were excluded: Three due to serious postoperative complications (two in group 2, and one in group 3), and six due to incomplete data collection (three in group 1, one in group 2 and two in group 3). Thus, a total of 81 patients were included in the data analysis. There were no significant differences in the demographics and surgical information among the three groups (P>0.05; Table I).

Pain intensity. Compared with group 1, VAS scores at rest and during coughing decreased in group 3 between 6 and 60 h following surgery (P<0.05. VAS scores between groups 1 and 2 were not significantly different (P>0.05; Fig. 1). Compared with group 2, VAS scores at rest and during coughing decreased in group 3 between 6 and 60 h following surgery (P<0.05). These results indicted that TAES at sham acupoints did not have any effect on the human body.

Percentage of Th cells. Representative binding patterns of the various $CD4^+$ T cell subsets are presented in Fig. 2. The percentage of Th_1 (intracellular antibodies against IFN-γ, 14.4%) and Th_{17} (intracellular antibodies against IL-17, 2.4%) cells in group 3 were significantly higher 72 h post-operation, as compared with groups 1 and 2 (9.2%; P<0.05; Fig. 2A and B). The percentage of Th_2 cells (intracellular antibodies against IL-4, 3.8%) in group 3 were significantly lower, as compared with in group 1 (8.3%) 72 h post-operation (P<0.05; Fig. 2A). There were no differences in the percentage of T_{reg} cells (intracellular antibodies against FoxP3) among all three groups (P>0.05; Fig. 2C).

Protein expression levels of Th cell-associated cytokines. Protein expression levels of IL-2 and IFN-γ were significantly increased at 72 and 120 h postoperatively in group 3, compared with group 1 (P<0.05; Fig. 3A and B). By contrast, IL-10 protein expression levels were significantly decreased at 72 h postoperatively in group 3, compared with group 1 (P<0.05; Fig. 3C). IL-17 protein expression levels were increased 120 h postoperatively in group 3, compared with group 1 (P<0.05; Fig. 3D). There were no significant differences in the protein expression levels of IL-4 and TGF-β among the three groups (P>0.05; Fig. 3E and F, respectively).

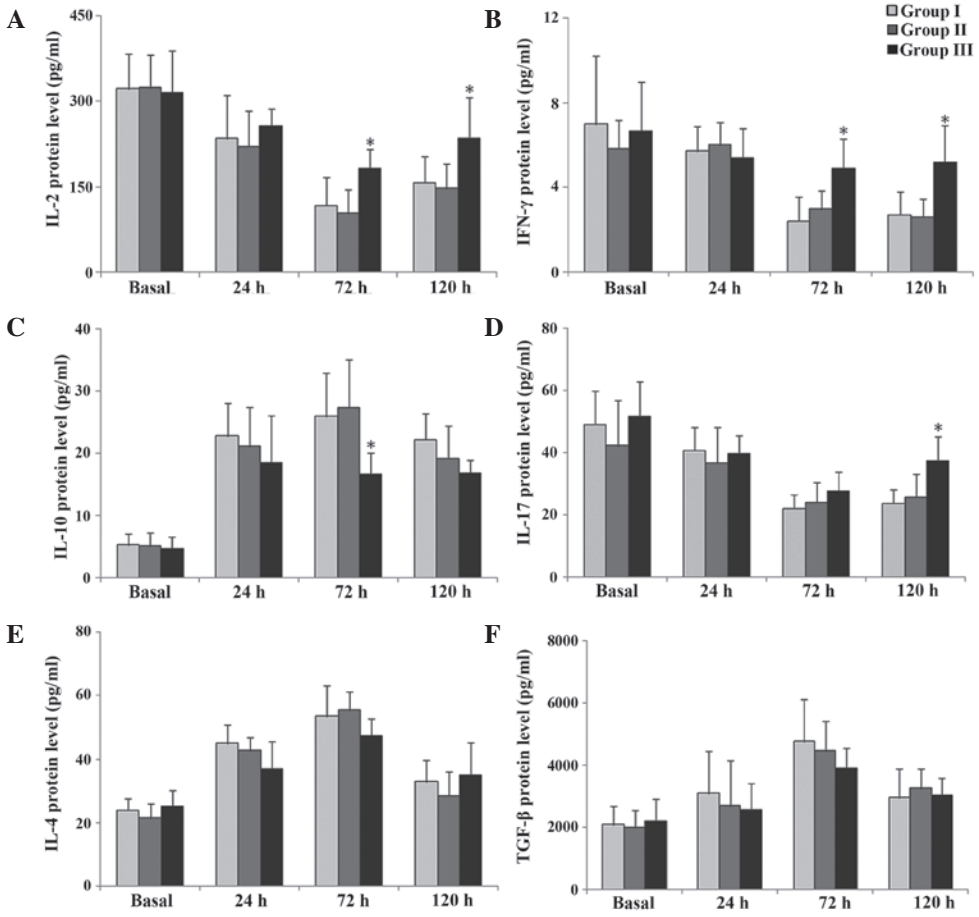

Figure 3. Protein expression levels of (A) IL-2, (B) IFN-γ, (C) IL-4, (D) IL-10, (E) IL-17 and (F) TGF-β prior to surgery (basal), and at 24, 72 and 120 h postoperatively, measured using ELISA. Data are presented as the mean ± standard deviation (n=27/group). *P<0.05 vs. group 1. IL, interleukin; IFN-γ, interferon-γ; TGF-β, transforming growth factor-β.

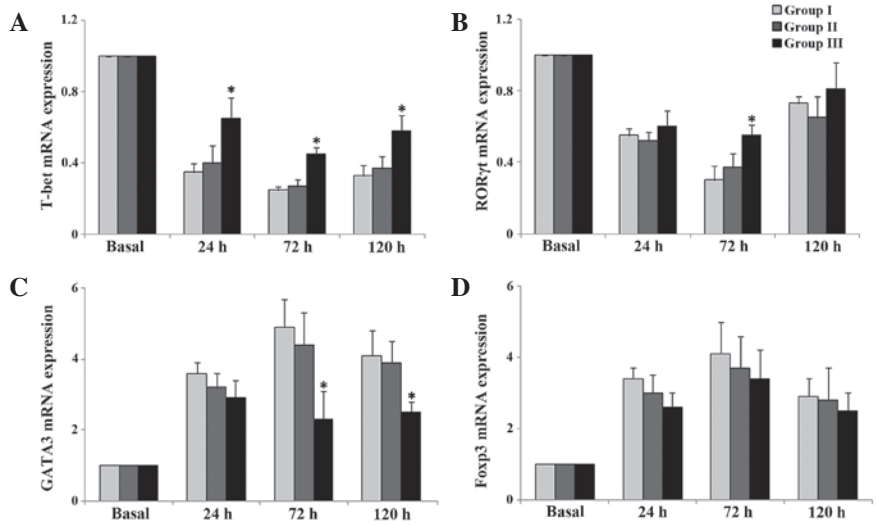

Figure 4. mRNA expression levels of (A) T-bet, (B) GATA3, (C) ROR-γt and (D) FoxP3 prior to surgery (basal), and at 24, 72 and 120 h postoperatively, measured using reverse transcription-quantitative polymerase chain reaction. GAPDH was used as an internal control and the relative mRNA expression levels for each cytokine were calculated. All of the samples were run in triplicate for each experiment. Data are presented as the mean ± standard deviation (n=27/group). *P<0.05 vs. group 1. GATA3, GATA-binding protein 3; ROR-γt, RAR-related orphan receptor-γt; FoxP3, forkhead box P3.

mRNA expression levels of Th cell-associated transcription factors. T-bet mRNA expression levels were significantly upregulated at 24, 72 and 120 h postoperatively in group 3, compared with group 1 (P<0.05; Fig. 4A). RORγt mRNA expression levels were significantly upregulated at 72 h postoperatively in group 3, compared with group 1 (P<0.05;

Fig. 4B). Conversely, GATA3 mRNA expression levels were downregulated at 72 and 120 h postoperatively in group 3, compared with group 1 ($P<0.05$; Fig. 4C). There were no significant differences in FoxP3 mRNA expression levels among the three groups ($P>0.05$; Fig. 4D).

Rescue analgesic, adverse events and hospital stays. Rescue analgesic demands were significantly lower in group 3, compared with group 1 ($P<0.05$). In addition, the incidence of postoperative nausea/vomiting and infection was significantly less in group 3, compared with group I ($P<0.05$; Table II). Hospital stays in group 3 (8.7±1.9 days) were marginally shorter, compared with groups 1 and 2 (10.3±1.5 and 11.5±1.6 days, respectively); however, there were no significant differences among the three groups ($P>0.05$).

Discussion

The present study detected an imbalance in the percentages of Th_1, Th_2, Th_{17} and T_{reg} cells, which was associated with downregulated expression levels of Th_1/Th_{17}-associated cytokines and transcription factors (IL-2, IFN-γ, IL-17, T-bet and RORγt), and upregulated expression levels of Th_2-associated cytokines and transcription factors (IL-10, GATA3), following thoracotomy of patients with lung cancer. TAES treatment was able to partially restore the imbalance in the various $CD4^+$ T-cell subsets, which may have contributed to attenuation of the postoperative immunosuppression in patients with lung cancer.

Postoperative pain is the most important consideration in the care of thoracic surgical patients. In previous studies, increased expression levels of endogenous catecholamines, as a result of surgical trauma, stress responses and pain, were associated with suppression of cellular immune responses and an increased probability of metastasis (23,24). It has previously been suggested that TAES is able to inhibit pain signals via the descending pathway and the dorsal horn cell and the spinothalamic tract, and by blocking the release of neurotransmitters, including β-endorphins, enkephalins and dynorphin (25). The results of the present study indicated that TAES was able to alleviate the postoperative pain of thoracic surgical patients with lung cancer. Numerous studies have suggested that the use of an effective analgesia may attenuate the occurrence of postoperative immunosuppression, which may explain why TAES was able to exert immunomodulatory effects.

Th_{17} and T_{reg} cells are the most recently discovered $CD4^+$ T-cell subsets. The percentage of Th_{17} cells and expression levels of RORγt were previously demonstrated to be decreased in the peripheral blood of patients with lung cancer (26); however, little is known about the postoperative balance of Th_{17} and T_{reg} cells in patients with lung cancer. In the present study, the balance of Th_1, Th_2, Th_{17} and T_{reg} cells was disrupted following thoracotomy of patients with lung cancer; thus suggesting that this imbalance may contribute to the postoperative immunosuppression commonly observed in these patients. Treatment with TAES increased Th_1 and Th_{17} cells, and decreased Th_2 cells, and this may have partially restored their balance. The results of the present study suggested that an imbalance in the numbers of the various $CD4^+$ T-cell subtypes in patients with lung cancer following surgery, may lead to postoperative immune depression, and that restoration of this imbalance may be the underlying mechanism of the TAES immunomodulatory effects.

Cytokines are important Th cell-polarization factors; therefore, the profiles of specific cytokines may be informative in the role of T cell dynamics in immune dysfunction. In our previous study, surgical trauma was associated with decreased expression levels of IL-2 and IFN-γ, increased expression levels of IL-4 and IL-10, and immunosuppression in a surgical trauma rat model (27). In the present study, the expression levels of Th_{17}-associated cytokines in patients with lung cancer were decreased following thoracotomy, which may have promoted an imbalance in numbers of Th_1, Th_2, Th_{17} and T_{reg} cells, and contributed to postoperative immunosuppression. Treatment with TAES increased the expression levels of IL-2, IFN-γ and IL-17, and decreased IL-10 secretion; thus suggesting that TAES was able to attenuate the postoperative immune impairment of patients with lung cancer via altering the expression of Th cell-associated cytokines. The results of the present study are in line with a previous study, in which electroacupuncture was able to improve surgery-suppressed immune function (28). Furthermore, this study reported that acupuncture and TAES were able to affect the immune system of patients undergoing major abdominal surgery (28).

Numerous studies have demonstrated that cytokine-mediated signals are predominantly transduced via specific transcription factors; for example, T-bet regulates the transcriptional initiation of Th_1 cytokines; GATA3 controls that of Th_2 cytokines; RORγt is an important transcription factor for the differentiation of Th_{17} cells; and Foxp3 is the master transcription factor in T_{reg} cells. In the present study, T-bet and RORγt mRNA expression levels were decreased and the mRNA expression levels of GATA3 were increased, following thoracotomy; thus suggesting that an imbalance in the expression of Th cell-associated transcription factors may have a role in the pathogenesis of postoperative immune suppression. Treatment with TAES increased T-bet and RORγt mRNA expression levels and decreased the mRNA expression levels of GATA3 in patients with lung cancer; thus suggesting that TAES is able to regulate the balance of Th cell-associated transcription factors.

In the present study, T_{reg} cells, and their associated cytokines and transcription factors, were not significantly altered following thoracotomy of patients with lung cancer. This may be due to the predominant use of lung cancer patients with early stage cancer: In previous studies, the percentages of T_{reg} cells in patients with early stage cancer increased marginally or did not increase at all postoperatively [preoperative vs. postoperative: Stage I (2.34 vs. 1.77%), stage II (2.72 vs. 1.94%)], whereas the postoperative T_{reg} percentage in patients with advanced stage (III+IV) cancer remained high (preoperative vs. postoperative: 1.61 vs. 3.52%) (29).

In the present study, acupoint stimulation occurred for 30 min, at a frequency setting of 2/100 Hz, and this model was selected for numerous reasons. First, a long duration of acupoint stimulation has been associated with enhanced patient discomfort, whereas a stimulation time that was too short had unclear therapeutic effects (30). In addition, low frequency-TAES triggered μ-and δ-opioid receptors, and β-endorphin production, whereas high frequency (100 Hz) stimulation was demonstrated to stimulate the κ-opioid receptor and resulted in the release of dynorphin (31,32).

Future studies should endeavor to optimize the TAES model in order to maximize its effects.

In the present study, patients treated with TAES required less analgesic treatment, which was associated with fewer side effects, including nausea/vomiting and infection. The reduced incidence of postoperative complications may have facilitated early recovery following thoracotomy of patients with lung cancer.

In conclusion, TAES was able to partially restore the post-operative immunosuppression of patients with lung cancer by altering the balance of Th_1, Th_2, Th_{17} and T_{reg} cells, and their associated cytokines and transcription factors. Therefore, TAES may provide a novel therapeutic intervention strategy for clinical immune dysfunction. Future studies should expand the application of acupuncture in clinical practice, in order to determine its immunomodulatory effects.

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