Type Two Cytokines Predominance of Human Lung Cancer and Its Reverse by Traditional Chinese Medicine TTMP

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Type 2 cytokines are usually predominant in tumor patients and associated with tumor progression. To explore whether reversing of type 2 predominance could be a promising strategy in tumor immunotherapy, PBMC of 35 lung cancer patients and 19 healthy subjects were prepared and subjected to be examined for cytokine secretion and gene expression. Tetra-Methylpyrazine (TTMP), extracted from a traditional Chinese medicinal herb which has been used in clinic to reverse the Th2 status of cancer patients in China, was added to PBMC culture. Determined by RT-PCR, the positive percentages of mRNA expression of type 1 cytokines (8.6% for IFN-γ and 11.4% for IL-2) were lower than those of type 2 cytokines (71.4% for IL-4, 60% for IL-6 and 80% for IL-10) in patients' PBMCs. The potential of gene expressing (measured as relative intensity to the ratio of β-actin) in the patients for type 1 cytokines was also in a low level (0.111 for IFN-γ, 0.119 for IL-2) in comparison with a relative high level for type 2 cytokines (0.319 for IL-4, 0.303 for IL-6 and 0.377 for IL-10). Meanwhile, both positive percentage and relative intensity of gene expression were lower for a type 1 cytokine-related transcription factor T-bet (31.4% and 0.142, respectively) than those for type 2 cytokine-related GATA3 (85.7% and 0.378, respectively). The blood serum levels of IFN-γ and IL-2 in the patients were slightly lower but not significantly when compared with healthy control. In contrast, the levels IL-4 and IL-6 in patients were significantly higher than those in healthy subjects by ELISA analysis. TTMP could enhance supernatant concentration and gene expression levels of IFN-γ, IL-2 and T-bet, but reduced those of type 2 cytokines. These results demonstrate that the lung cancer patients had a predominant expression of type 2 cytokines and TTMP could reverse the type 2 dominant status, which might offer an alternative therapeutic regime for lung cancer patients. *Cellular & Molecular Immunology*. 2004;1(1):63-70.

Key Words: Th2 cytokine, predominance, lung cancer, Chinese medicine, Tetra-Methylpyrazine

Introduction

Recent research has demonstrated that cytokine network is involved in the local immunity against tumors (1-3). In both murine models and human studies, T lymphocytes have been found to express two distinct cytokine profiles. Type 1 or Th1 cells are principal effectors of cell-mediated immune response by secreting IL-2 and IFN-γ, which benefit anti-tumor immunity. Type 2 or Th2 cells, on the other hand, produce the humoral immune response-related cytokines IL-4, IL-5, IL-6 and IL-13, which are involved in suppressing anti-tumor immunity (4-6). The imbalance of Th1/Th2 status may cause infection (7), autoimmune disorders (8) and allergic diseases (9). Recent studies have demonstrated that Th2 type cytokine expression is predominant in many kinds of tumor patients, suggesting that such kind of cytokines may mediate immuno-suppression for the Th1-dominant immune response to tumor antigens. One aim of present study is to detect the expressing levels of Th1 and Th2 type cytokines in sera and culture supernatants, and the relevant transcription factors from peripheral blood mononuclear cells (PBMCs) of lung cancer patients.

Reversing of type 2 dominant status is thought to be a promising strategy, based on which a variety of modulators have been tested *in vivo* such as cytokines (10, 11), oligo-dexynucleotides (12, 13), extracts from fungi or bacteria (14, 15), metal composite (16) and Japanese-Chinese medical herbal (17). In order to investigate the possibility of using Tetra-Methylpyrazine (TTMP), extracted from traditional Chinese medicinal herb and used in clinic intravenously in

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Abbreviations: IFN-γ, interferon gamma; STAT, signal transducer and activator of transcription; IL-2, 4, 6, 10, interleukin 2, 4, 6, 10; Th2, T helper type 2 cells; TTMP, Tetra-Methylpyrazine.

China, for reversing type 2 status of lung cancer, TTMP was added to PBMC culture and the levels of Th1/Th2 type cytokines were examined. In the present study, we at first time reported that lung cancer patients represented an obvious type 2 cytokine predominance, traditional Chinese medicine TTMP might reverse the type 2 predominant status *in vitro*.

Materials and Methods

Cancer patients and healthy subjects

The 35 lung cancer patients (21 men, aged 39-70; and 14 women, aged 36-62) were recruited from Shandong University Qilu Hospital, Jinan City, China and were new to this study. The patients fulfilled criteria for lung cancer and the clinical characteristics of patients were summarized in Table l. The lung cancer patients were histocytologically proven. The cancer histology included 9 middle differrentiation squamous carcinomas patients, 8 poorly differentiation squamous carcinomas patients, 4 middle differentiation adenocarcinomas patients, 7 poorly differentiation adenocarcinomas patients and 8 small cell lung cancer patients. The cancer stage ranged from I-IV, i.e., 12 patients had stage I-II, and 23 patients had stage III-IV cancer. Exclusion criteria for patients were the following: pregnancy, severe infection, simultaneous use of corticosteroids, previous TTMP treatment or any other immunotherapy and chemotherapy. Nineteen healthy subjects (11 men and 8 women, aged 21-47 years) served as controls. No significant difference was observed between the two groups with regard to age, male/female ratio. The Ethical Committee of Shandong University School of Medicine carried out the study after the approval, and informed consent was obtained from each patient prior to participation in the study and blood sampling.

Table 1. *Patients Characteristics.*

Characteristics	Number of Patients
Median age (range)	54.6(36-70)
Gender	
Male	21
Female	14
Histology	
Middle differentiation squamous carcinomas	9
Poorly differentiation squamous carcinomas	7
Middle differentiation Adenocarcinomas	4
Poorly differentiation Adenocarcinomas	7
Small cell lung cancer	8
TNM stage	
I	2
II	10
III	11
IV	12

Isolation of mononuclear cells from blood samples and treatment with traditional Chinese medicine in vitro

Total of 20ml peripheral blood samples was collected from each subject and divided into two tubes: 15ml of blood was heparinized for isolation of PBMCs and another 5ml was agglutinated for sera. PBMCs were immediately separated by Ficoll-Hypaque gradient according to the procedure described by Bicalho et al. (18). The cells were washed twice in RPMI1640 medium supplemented with 10% fetal calf serum (FCS) and gentamycin (lμg/ml). Cell numbers were determined by light microscopy and the viability was assessed by the 0.25% trypan blue exclusion technique. Human PBMCs (2×10^6cell/ml) were cultured for 48hrs in duplicates with or without TTMP (10mg/ml) (Fourth Pharmaceutical Company of Beijing, Beijing, China). The culture supernatants and cultured cells were carefully collected and frozen at -80°C until use, respectively.

Table 2. *Sequence of primers and condition for RT-PCR.*

Transcription	Sequence	Annealing temperature (^{0}C)	Cycle	Product size (bp)
IFN-γ	(F) 5'ATGAAATATACAAGTTATATCTTGGCTTT3' (R) 5'GATGCTCTTCGACCTCGAAACAGCAT3'	58	33	494
IL-2	(F) 5'ATGTACAGGATGCAACTCCTGTCTT3' (R) 5'GTTAGTGTTGAGATGATGCTTTGAC3'	58	33	458
IL-4	(F) 5'ATGGGTCTCACCTCCCAACTGCT3' (R) 5'CGAACACTTTGAATATTTCTCTCTCTCAT3'	58	33	456
IL-6	(F) 5'CCGAATTCATGATTGACAAACAAATTCCGG3' (R) 5'CGCGGATCCTTACATTTGCCGAAGAG3'	58	33	511
IL-10	(F) 5'ATGCCCCAAGCTGAGAACCAAGACCCA3' (R) 5'GTTTCGTATCTTCATTGTCAT3'	58	33	249
T-bet	(F) 5'CCTCGCACCTGGAGCTGGCTG3' (R) 5'TTATCAGTTGGGAAAATAGTTA3'	58	33	334
GATA3	(F) 5'GAATGCCAATGCGGACCCTG3' (R) 5'CTAACCCATGGCGGTGACCA3'	58	33	343
β-actin	(F) 5'GTGGGCGCCCAGGCACCA3' (R) 5'CTCCTTAATGTCACGCACGATTT3'	58	33	520

(F) Forward primer; (R) Reverse primer

Table 3. *mRNA expression of two types of cytokines and their up-stream transcription factors in PBMCs from patients with lung cancer.*

Pathology	Sample no.	IFN-γ		IL-2		T-bet	
		Positive	Intensity	Positive	Intensity	Positive	Intensity
Middle differentiation Squamous carcinomas	9	0	0.112±0.041	1	0.124±0.021	2	0.136±0.042
Poorly differentiation Squamous carcinomas	7	1	0.116±0.018	1	0.113±0.014	2	0.131±0.038
Middle differentiation Adenocarcinomas	4	0	<0.1	0	<0.1	2	0.149±0.053
Poorly differentiation Adenocarcinomas	7	1	0.115±0.013	1	0.109±0.011	2	0.144±0.048
Small cell lung cancer	8	1	0.105±0.012	1	0.118±0.015	3	0.138±0.042
Total(%)(x±s)	35	3(8.6%)	0.111±0.011	4(11.4%)	0.119±0.016	11(31.4%)	0.142±0.043

Pathology	Sample no.	IL-4		IL-6		IL-10		GATA3	
		Positive	Intensity	Positive	Intensity	Positive	Intensity	Positive	Intensity
Middle differentiation Squamous carcinomas	9	7	0.221±0.079	7	0.224±0.058	6	0.314±0.088	7	0.362±0.092
Poorly differentiation Squamous carcinomas	7	6	0.342±0.099	5	0.313±0.091	7	0.422±0.101	7	0.337±0.083
Middle differentiation Adenocarcinomas	4	2	0.276±0.064	2	0.251±0.064	3	0.356±0.094	3	0.441±0.105
Poorly differentiation Adenocarcinomas	7	5	0.317±0.076	3	0.327±0.087	5	0.429±0.104	6	0.417±0.094
Small cell lung cancer	8	5	0.325±0.087	4	0.318±0.079	7	0.338±0.081	7	0.297±0.067
Total(%)(x±s)	35	25(71.4%)	0.319±0.079	21(60%)	0.303±0.071	28(80%)	0.377±0.093	30(85.7%)	0.378±0.098

The positive percentage and relative intensity of cytokine mRNA levels in PBMCs were determined by RT-PCR. All the samples were determined immediately after isolation of PBMCs. Each sample was triplicate in each experiment.

Table 4. *Comparison of patients with healthy individuals in serum levels of type 1 and type 2 cytokines (pg/ml).*

	IFN-γ	IL-2	IL-4	IL-6	IL-10
Patients	97.25 ±57.08	72.86 ±49.98	116.80 ±28.70	440.12 ±111.22	60.06 ±31.11
Healthy	131.40 ±70.17	97.22 ±37.85	51.40 ±14.98	47.04 ±18.22	36.69 ±16.51
p value	>0.05	>0.05	<0.01	<0.01	>0.05

The serum cytokine levels were determined by ELISA. All the samples were determined within 3 months after collection. Each sample was triplicate in each experiment.

Detection of cytokines in the serum and culture supernatant

The cytokine levels were determined by ELISA according to the Manufacture's instructions using commercially available kits from PharMingen. The sensitivities of these assays were as follows: IFN-γ>5pg/ml, IL-2>10pg/ml, IL-4>20pg/ml, IL-6>4pg/ml, IL-10>5pg/ml. All the samples were determined within 3 months after collection. Positive and negative controls were included in the assay.

Detection of cytokine mRNA expression by RT-PCR

PCR primers for detecting mRNAs of IFN-γ, IL-2, IL-4, IL-6, IL-10, T-bet, GATA3 and β-actin were designed by us to be 18-24 nucleotides long and to have a 100% homology with the particular regions of the genes according to gene sequences. The gene sequences were obtained using the OLIGO Primer Analysis Software, Version 5.0 (NBA, Software and Research Services for Tomorrow's Discoveries, National Biosciences, Plymouth, MN). PCR oligomers were synthesized by a DNA/RNA synthesizer (Applied Biosystems) at the University of Science & Technology of China Oligonucleotide Synthesis Facility. Primer sequences and transcription conditions were listed in Table 2.

The RT-PCR method was used as previously described (19). Briefly, RNA was extracted from peripheral blood mononuclear cells (PBMCs) using the acid-guanidinium phenol-chloroform method. RT-PCR was performed using RNA PCR kit (Perkin-Elmer, Norwalk, CT). Cellular RNA (1μg) was reversedly transcribed into cDNA in a reaction mixture containing 5mM $MgCl_2$, 1mM dNTP, 2.5μM oligo (dT) primer, 1U RNase inhibitor, and 2.5U reverse transcriptase. After incubation at 37°C for 60 min, the reaction was terminated by heating at 95°C for 5 min. PCR was performed using the sense/antisense primers listed above. The PCR reaction buffer (25μl), consisting of 2mM $MgCl_2$, 0.5μM of each primer, and 1U Ampli *Taq* DNA polymerase (5μl of each reverse-transcriptase solution), was added to an amplification tube. PCR was run for 33 cycles. Each cycle consisted of 95°C for l min, 58°C for l min, and 72°C for l min. Twenty-microliter aliquots of the amplified product were fractionated on a 2% agarose gel and visualized by ethidium bromide staining. The band intensity of ethidium bromide fluorescence was measured using NIH

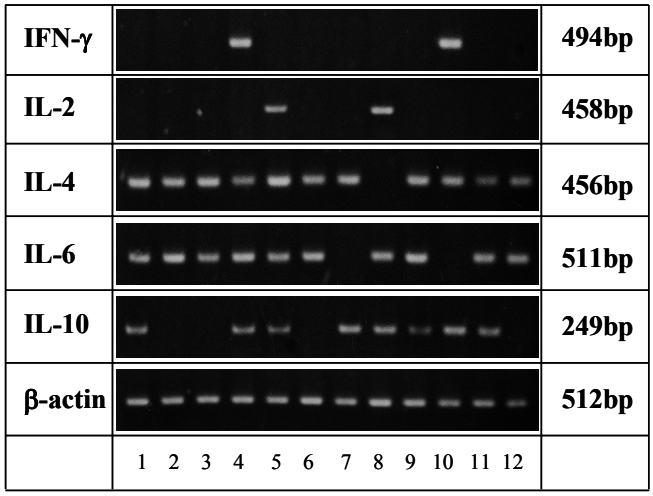

Figure 1. The expression of Th1/Th2 type cytokine gene in PBMCs of lung cancer patients. Cytoplasmic total RNA was extracted from peripheral blood mononuclear cells (PBMCs) from 12 of 35 lung cancer patients. RT-PCR was performed using RNA PCR kit (Perkin-Elmer, Norwalk, CT). Twenty-microliter aliquots of the amplified product were fractionated on a 2% agarose gel and visualized by ethidium bromide staining. All the samples were determined within 3 months after collection. All experiments were repeated at least three times and each sample was triplicate in each experiment.

Image Analysis Software Ver 1.61 (National Institutes of Health, Bethesda, MD). The relative intensity (RI) of each band was determined with the use of the ratio to β-actin.

Statistical analysis

The serum and supernatant cytokine levels were presented as means and standard deviations. To determine whether significant differences existed in cytokine levels after treated by TTMP, Students test was performed. Differences were considered significant when the p value was <0.05. All experiments were repeated at least four times and each sample was triplicate in each experiment.

Results

Predominance of type 2 cytokine expression in lung cancer patients

Total RNA from PBMCs of 35 lung cancer patients and 19 healthy subjects were prepared, and the cytokine mRNA expression profiles from these cells were determined by RT-PCR (Figure 1). To exclude the possibility of carry-over contamination, reactions containing all RT-PCR reagents including cytokine PCR primers without sample RNA were used as negative controls. No contamination was detected. The mRNA expressions of type 1 cytokines (IFN-γ and IL-2) and their up-stream transcription factor (T-bet), and type 2 cytokines (IL-4, IL-6 and IL-10) and their up-stream transcription factor (GATA3) in PBMCs were analyzed. As shown in Table 3, the percentages of mRNA expression of type 1 cytokines (8.6% for IFN-γ and 11.4% for IL-2) in freshly-isolated patients' PBMCs were nearly same as those of type 1 cytokines of the healthy (data not shown), but remarkably lower than those of type 2 cytokines (71.4% for IL-4, 60% for IL-6 and 80% for IL-10) of lung cancer patients, which were negative in the healthy (data not shown). In addition, the expressing capacity (measured as relative intensity to the ratio of β-actin) of patients for type 1 cytokines was in a low level (0.111 for IFN-γ, 0.119 for IL-2), but constitutively in a relatively high level for type 2 cytokines (0.319 for IL-4, 0.303 for IL-6 and 0.377 for IL-10). Meanwhile, the common transcription factors for type 1 or type 2 cytokines, T-bet or GATA3, were measured by detecting the gene expression. As shown in Table 3, both positive percentage and relative intensity were lower in T-bet (31.4% and 0.142, respectively) than those in GATA3 (85.7% and 0.378, respectively). These results demonstrated that lung cancer patients manifested a predominantly expressed type 2 cytokines at both gene regulating level (transcription factor) and gene expression level, although their type 1 cytokine status did not obviously changed if compared with the healthy.

In order to further confirm the predominant status of type 2 cytokines in lung cancer patients, ELISA method was performed to analyze the levels of both types of cytokines in serum of lung cancer patients. The ELISA results were shown in Table 4. The levels of type 1 cytokines, IFN-γ and IL-2, in patients were slightly lower but not significant when compared with those in healthy control. In contrast, the levels of type 2 cytokines, IL-4 and IL-6 (but not IL-10), in patients were significantly higher than those in healthy subjects. These results further demonstrated that type 2 cytokine predominance was present in lung cancer by highly secreting type 2 cytokines from PBMCs.

Reverse of type 2 cytokine predominant status of lung cancer patients after treatment with traditional Chinese medicine TTMP

Tetra-Methylpyrazine (TTMP) is an extract from traditional Chinese herbal medicine Chuangxiong, which has been

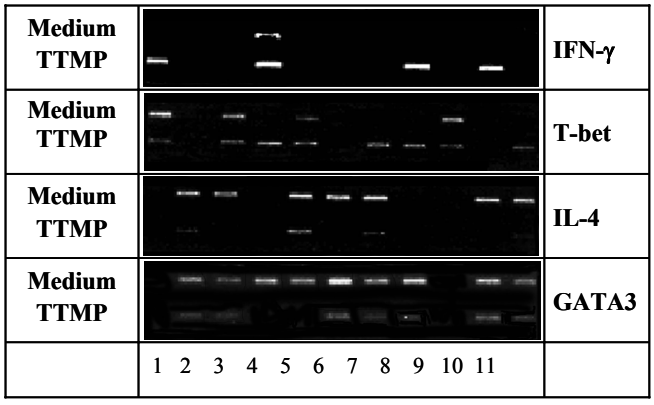

Figure 2. Reversing effects of traditional Chinese medicine TTMP on gene expression of cytokines in PBMCs from lung cancer patients. Cytoplasmic total RNA was extracted from peripheral blood mononuclear cells (PBMCs) from 11 of 35 lung cancer patients incubated with or without TTMP. RT-PCR was performed using RNA PCR kit (Perkin-Elmer, Norwalk, CT). Twenty-microliter aliquots of the amplified product were fractionated on a 2% agarose gel and visualized by ethidium bromide staining. All the samples were determined within 3 months after collection. All experiments were repeated at least three times and each sample was triplicate in each experiment.

used clinically in a variety of diseases to protect liver, kidney, brain and heart from injury for thousands years, based on traditional Chinese medicine theory, Yi-Qi-Huo-Xue (strengthening and activating blood). In order to further survey the possibility of using TTMP in lung cancer patients, we observed the effects of TTMP on regulating cytokine and transcription factor gene expression of PBMCs *in vitro*. As shown in Table 5 and Figure 2, TTMP did not obviously influence the cytokine (IFN-γ and IL-4) and transcription factor (T-bet and GATA3) gene expression in normal subject, but did increase the gene expression of type 1 cytokine and its transcription factor (IFN-γ and T-bet) in lung cancer patients: the positive percentage and relative intensity of IFN-γ extremely increased from 8.6% (3/35) to 42.8% (15/35) and 0.111 to 0.278, respectively, those of T-bet from 31.4% (11/35) to 85.7% (30/35) and 0.142 to 0.293, respectively. Interestingly, TTMP obviously but not extremely as observed in type 1 cytokines, reduced the percentage and relative intensity of IL-4 from 71.4% (25/35) to 40% (14/35) and 0.319 to 0.189, respectively. GATA3 expressing level was also reduced similarly as IL-4. In addition to detecting mRNA levels of cytokines by RT-PCR analysis, we also examined the supernatant concentrations of cytokines secreted by cultured PBMCs after stimulation with TTMP by ELISA method. As shown in Table 6, both types of cytokines (IFN-γ, IL-2, IL-4, IL-6, IL-10) in culture of PBMCs from the healthy subjects did not change in the present of TTMP. Similarly as what observed in PBMC gene expression of lung cancer patients, type 1 cytokines (IFN-γ and IL-2) significantly increased and type 2 cytokines (IL-4, IL-6, but not IL-10) significantly decreased after stimulation with TTMP, indicating that TTMP was able to reverse the type 2 predominant status of lung cancer patients.

Discussion

The Th1 and Th2 cytokine patterns have now been implicated in several immune responses concerning infections, allergy and antoimmunity (20). Recent clinical information also suggests that Th1 response is suppressed and Th2 response is elevated systemically in tumor patients, suggesting that Th2 cytokines may mediate immuno-suppression (21, 22). It is generally considered that the immune response to malignantly transformed cells requires the collaboration of cytokines produced by T cells. Th1 cytokines appear to have a protective function, whereas Th2 cytokines seem to favor tumor growth (23). Pellegrini et al. (24) have described an increased expansion in Th2 cells or Th2 cell clones and a decreased function in Th1 cells in colon cancer patients. A shift from Th1 to Th2 cell function has been observed in several animal tumor models (3-5) and also in human tumors (6-8). Th2-mediated immuno-suppression reduced the protective cellular immunity and

Table 5. *Reverse of type 2 cytokine gene expressing by traditional Chinese medicine TTMP in PBMCs from patients with lung cancer.*

	Sample no.	IFN-γ		T-bet		IL-4		GATA3	
		Positive	Intensity	Positive	Intensity	Positive	Intensity	Positive	Intensity
Healthy									
PBMCs	19	0	<0.1	11	0.132±0.013	0	<0.1	15	0.127±0.013
PBMCs + TTMP	19	3	0.121±0.011	13	0.141±0.013	0	<0.1	13	0.135±0.014
Patient									
PBMCs	35	3(8.6%)	0.111±0.011	11(31.4%)	0.142±0.013	25(71.4%)	0.319±0.029	30(85.7%)	0.378±0.038
PBMCs+ TTMP	35	15(42.8%)	0.278±0.031*	30(85.7%)	0.293±0.031*	14(40%)	0.189±0.019*	24(68.6%)	0.201±0.019*

*$p<0.05$. The positive percentage and relative intensity of cytokine mRNA levels in PBMCs were determined by RT-PCR. All the samples were determined with 3 months after collection. All experiments were repeated at least four times and each sample was triplicate in each experiment.

Table 6. *Reversing effects of traditional Chinese medicine TTMP on cytokine secretion in PBMCs from patients with lung cancer (pg/ml).*

	IFN-γ	IL-2	IL-4	IL-6	IL-10
Healthy					
PBMCs	111.56±68.37	105.45±51.68	56.50±13.53	50.39±32.76	34.56±19.07
PBMCs + TTMP	113.12±64.79	167.27±102.87	48.64±13.95	45.74±25.52	33.47±18.25
Patient					
PBMCs	24.63±6.29	63.00±21.12	114.72±27.15	1412.77±263.20	99.15±23.15
PBMCs + TTMP	48.57±13.71*	122.19±29.33*	59.31±14.03*	912.13±183.11*	72.19±19.20

*$p<0.05$. The cytokine levels in culture supernatant of PBMCs were determined by ELISA. All the samples were determined with 3 months after collection. All experiments were repeated at least four times and each sample was triplicate in each experiment.

was found to be associated with tumor progression (4). Until recently, Th2 predominant status in lung cancer patients has also been observed by at least six groups (25-30): the serum level (26, 28), the supernatant concentration from cultured PBMCs (27) and tumor infiltrating lymphocytes (TIL) (25), the percentages of intracellular positive Th cells (27, 29), mRNA expressing levels of PBMCs and TIL (25, 30) were extremely upregulated in productions of IL-4 and IL-10 in lung cancer patients. In the present study, the role of type 2 T cells or their cytokine products were investigated in 42 lung cancer patients by detecting blood serum, secreting supernatant and gene expression of PBMCs from patients. Our data further confirmed that Th2-dominant immune status might occur in lung cancer patient, which was supported by the finding that IL-4, IL-6 and IL-10 levels, but not IFN-γ and IL-2, were significantly higher in the serum, secreting supernatant or transcripts produced by PBMCs from lung cancer patients.

IL-4 is a pleiotropic type 2 cytokine that has been found to have inhibitory effects on anti-tumor immune response (31, 32). IL-4 directs the development of Th2 cells both *in vitro* and *in vivo*, down-regulates IFN-γ production in Th1 cells (33), inhibits the production of IL-12 by monocytes (34) and IFN-γ production in human monocytes (35). IL-10 also possesses several properties that may be inhibitory to the generation of anti-tumor immunity. IL-10 inhibits a broad array of immune parameters, including proinflammatory cytokine production by macrophages (36), antigen-presentation function (37), T lymphocyte proliferation (38) and Th1 cytokine production (29). Both IL-4 and IL-10 are key cytokines for the inhibition of Th1 cytokine response and the development of the Th2 cytokine response. In our data presented here, the expression and production of Th1 cytokines were inhibited with a concomitant predominant state of Th2 cytokines produced by PBMCs from lung cancer patients, which might at least partly explain the suppressed immune response against tumor in these patients.

Much effort has recently been placed in elucidating the pathways used by each type of cytokines to mediate their action. These studies have revealed that cytokine-mediated signals are primarily transduced by the Jak-Stat signaling cascade (39, 40). STAT4 and its up-stream, T-bet, controlled transcriptional initiation of type 1 cytokines, in contrast, STAT6 and its up-stream, GATA3, controlled that of type 2 cytokines, the underlying mechanisms well explained the type 2 predominance and pathogenesis of type 2-biased disease (41, 42). In another words, STAT6-GATA3 activation or STAT4-T-bet inactivation are possibly necessary steps for progression of type 2-biased diseases such as tumor. GATA3 is expressed in the course of Th2 differentiation through pathways that probably involve the IL-4 dependent activation of STAT6, but is undetectable in Th1 cells (43-45). In addition, GATA3 has been shown to directly regulate IL-5 and IL-13 expression and thus appears to play a more pivotal role in regulating Th2 cytokines (46, 47). We found that the transcription factor GATA3 was the dominant species that was present in the RT-PCR products and over-expressed in PBMCs of lung cancer patient, indicating that expression of transcription factor GATA3 was upregulated and T-bet was down-regulated. The results indicated that Th2-biased status in lung cancer was correlated with GATA3 over-activation, same as what happened in Th2-biased asthma (40).

Tetra-Methylpyrazine (TTMP) is an extract from traditional Chinese herbal medicine, ChuangXiong, which has been used clinically for thousands years, and in animal models for searching mechanisms in treatment of various diseases such as liver injury (48, 49), kidney disease (50), heart (51-53), brain (54), and vascular diseases (55, 56), based on traditional Chinese medicine theory, Yi-Qi-Huo-Xue (strengthening and activating blood). In order to investigate the possibility to use TTMP as a therapeutic regime of reversing type 2 status of lung cancer, TTMP was added to PBMC culture and the levels of Th1/Th2 type cytokines were examined. In the results presented, the secreting and transcripting levels of type 2 cytokines, IL-4, IL-6, IL-10, and their up-stream transcription factor, GATA3, significantly reduced during treatment with TTMP, on the contrary, the levels of IFN-γ, IL-2 and T-bet increased significantly. IFN-γ is a critical cytokine, dominantly released by Th1 and NK cells, and plays a major role in anti-tumor cell-mediated immune response. IL-4 and IL-10, as the natural antagonists, suppress the function and production of IFN-γ. So the reversing effects on type 2 cytokines and promoting effects on type 1 cytokines of TTMP reveal that TTMP, a classic traditional Chinese herbal medicine, will be a promising candidate for treating type 2-biased diseases including tumor, allergic disease and intracellular pathogenic infections. The clinical trial for treating lung cancer with TTMP and its immunological mechanisms are under investigation in China.

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