Enhanced circulating ILC2s and MDSCs may contribute to ensure maintenance of Th2 predominant in patients with lung cancer

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Abstract. Group 2 innate lymphoid cells (ILC2s) were demonstrated to be involved in the initiation and coordination of type 2 T helper cell (Th2) responses. Myeloid-derived suppressor cells (MDSCs) have received a great deal of attention for their role in creating an immunosuppressive microenvironment in cancer-bearing hosts. However, the contributions of ILC2s in the occurrence and development of lung cancer, and the association between ILC2s and Th2 or MDSCs in lung cancer remain to be elucidated. In the present study, 36 patients newly diagnosed with lung cancer based on the guidelines of the International Union Against Cancer Tumor Node Metastasis were included. The frequencies of ILC2s and MDSCs in peripheral blood mononuclear cells were determined, and the mRNA expression levels of ILC2s or Th2-related transcription factors and cytokines, and MDSCs-related products were assessed. The results demonstrated that the frequencies of the circulatory ILC2s and MDSCs were enhanced in lung cancer patients, as were ILC2-related transcription factors and cytokines in peripheral blood. A positive correlation was identified between the Th2-dominated phenotype and the expression levels of ILC2s-associated cytokines or transcription factors. In addition, increased autophagy related 1 was closely associated with Th2-associated transcription factors. It was demonstrated that ILC2s and MDSCs were clearly upregulated and accompanied by a predominant Th2 phenotype in patients with lung cancer; this may lead to new immunotherapy approaches for lung cancer based on the associated metabolites and cytokines.

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Introduction

Lung cancer remains a daunting health problem with more than 1.1 million mortalities worldwide attributed to lung cancer annually (1). Although there is growing evidence that the immune system has the potential to protect against malignancies, the precise mechanisms of immune modulation in lung cancer patients remain elusive, particularly that exerted by the innate immune system, which is considered to be the primary response against tumor growth and development. Previous studies (2,3) have indicated that the immune system may be involved in the protection and promotion of carcinogenesis.

Innate lymphoid cells (ILCs) are a heterogeneous group of cell types that serve a role in innate immune responses and which have received a great deal of attention. ILCs are categorized into three distinct populations based on the surface markers they express, the transcription factors that regulate their development and function and the effective cytokines they can produce (4,5). Group 2 ILCs mainly produce interleukin (IL)-13 and IL-5, and express the transcription factors retinoic acid receptor related orphan receptor α (RORα) or GATA binding protein 3 (GATA3; 4,6,7). These cells respond to IL-25 and IL-33 *in vitro* due to IL-17 receptor (R) B or ST2 expression on their membrane (8,9).

Myeloid-derived suppressor cells (MDSCs) are defined as a heterogeneous population of activated immature myeloid cells characterized by a morphological mixture of granulocytic and monocytic cells. However, they lack the expression of cell-surface markers that are specific to the fully differentiated monocytes, macrophages, or dendritic cells. In mice, granulocytic MDSCs possess a $CD11b^+Ly6G^+Ly6C^{low}$ phenotype, whereas MDSCs with monocytic morphology are $CD11b^+Ly6G^-Ly6C^{high}$; the two phenotypes exhibit different functions in cancer and autoimmune diseases. By contrast, the human MDSCs are traditionally defined as $CD14^-CD11b^+CD33^+CD15^+$ cells or cells that express the CD33 marker but lack the expression of markers of mature myeloid and lymphoid cells and the major histocompatibility complex class-II molecule human leukocyte antigen-antigen D related (10). The identification and isolation of human MDSC subsets have been challenging due to the heterogeneous characteristics of these immature cells and accumulating data suggest a significant diversity in the MDSC

subsets identified in different human cancers (11). The frequency of each MDSCs subset appears to be influenced by the type of cancer (12). MDSCs are suspected of serving a crucial role in local and systemic tumor development, providing a microenvironment in which tumor cells can proliferate, expand, acquire new mutations and escape host immunosurveillance (13). Elevated numbers of MDSCs in many cancer patients have been demonstrated, including patients with lung cancer (14-16), and their inhibitory role in adaptive immunity via their suppression of T cell activation is well established (17-20). However, the influence of MDSCs on Th1/Th2 balance is little known in lung cancer. In previous studies of the authors (21,22), it was identified that there were markedly enhanced ILC2s and a predominant Th2 phenotype in patients with gastric cancer. However, it remains controversial whether these ILC2s serve a role in the progression of lung cancer. The present study was designed to investigate the changes in the frequency of ILC2s and the expression levels of ILC2s-associated factors in patients with lung cancer, and to analyze the association between circulating ILC2s, or associated factors, and Th1/Th2 immune imbalance, with the aim of understanding the significance of ILC2s in lung cancer.

Materials and methods

Patients and healthy controls. A total of 36 patients (26 male, 10 female), newly diagnosed with lung cancer based on the guideline of International Union Against Cancer Tumor Node Metastasis (23) were included in the current study. These patients ranged between 43 and 70 years of age (mean 52.35 years) and 30 healthy subjects, age- and sex-matched to the patients, were studied as the controls. None of the patients had been treated preoperatively or had a history of autoimmune disease, and none of the healthy controls had a prior history of cancer or any chronic inflammation. The present study was approved by the Ethical Committee of the Affiliated People's Hospital of Jiangsu University (Zhenjiang, China), and written informed consent was obtained from all individuals.

Cell preparation. Peripheral blood samples were collected from lung cancer patients and healthy controls. Plasma samples were frozen at -80°C immediately following centrifugation at 500 x g and at 4°C until use. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Hypaque centrifugation at 500 x g and at 4°C. The cell suspensions were divided into two equal aliquots, one was immediately used for experiments, and then 1 ml TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added to the other aliquot, which was stored at -80°C for extracting total RNA.

Flow cytometric quantification of ILC2s and MDSCs. ILC2s population was defined as lineage (CD2, CD3, CD14, CD16, CD19, CD56 and CD235a) and lineage⁻/inducible T-cell costimulator (ICOS)⁺/IL-17RB⁺; MDSCs population was defined as lineage⁻/HLA-DR⁻/CD33⁺. For flow cytometric quantification of ILC2s: PBMCs were stained with a fluorescently labeled antibody mix (FITC-conjugated anti-human CD2, CD3, CD14, CD16, CD19, CD56 and CD235a; 22-7778-72; 1:50; eBioscience, Inc.; Thermo Fisher Scientific, Inc.), with Allophycocyanin- (APC) -conjugated anti-human

ICOS (FAB6975A; 1:200; R&D Systems, Inc., Minneapolis, MN, USA) and PerCP-conjugated anti-human IL-17RB (FAB1207C; 1:200; R&D Systems, Inc.). For flow cytometric quantification of MDSCs: PBMCs were stained with antibody mix (FITC-conjugated anti-human CD2, CD3, CD14, CD16, CD19, CD56 and CD235a; 22-7778-72; 1:50; eBioscience, Inc.; Thermo Fisher Scientific, Inc.) with PE-conjugated anti-human HLA-DR (FAB4869P; 1:200; R&D Systems, Inc.) and PE conjugated anti-human CD33 (15-0339; 1:100; eBioscience Inc.; Thermo Fisher Scientific, Inc.); incubated at room temperature for 30 min. Following incubation, the samples were washed with phosphate buffered saline (PBS) and the pellets were resuspended in 250 μl PBS. The isotype control antibody was used in all cases. The labeled cells were analyzed with an Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) using FlowJo software version 7.6 (Tree Star, Inc., Ashland, OR, USA).

RNA extraction, cDNA synthesis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from individual PBMCs and 500 ng RNA was reverse transcribed using PrimeScript® RT reagent kit Perfect Real Time (Takara, Bio, Inc., Ostu, Japan), according to the manufacturer's protocols. For RT-qPCR, reverse transcribed cDNA (1 μl) was amplified by RT-PCR using the SYBR Green Premix EX Taq kit (Takara Bio, Inc.). The reaction was performed in a 10 μl solution containing 1 μl DNA template, a forward primer (10 μM), a reverse primer (10 μM), 2X SYBR Premix EX Taq II (1X, final concentration) and distilled water. Then the reaction was carried out as follows: 95°C 5 min then 40 cycles of denaturation at 95°C for 10 sec, annealing at 72°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 5 sec. Each sample was analyzed in duplicate with the CFXA96 Cycler (Thermo Fisher Scientific, Inc.) and the expression data for each target gene was then normalized relative to β-actin. All primer sequences are presented in Table I.

ELISA. The protein expression levels of IL-33, IL-13 and IL-5 in plasma were measured by an ELISA kit following the manufacturer's protocols (IL-33; BMS2048; eBioscience Inc.; Thermo Fisher Scientific, Inc.; IL-13; BMS231TNST; eBioscience Inc.; Thermo Fisher Scientific, Inc.; and IL-5; BMS278TNSTCE; eBioscience, Inc.; Thermo Fisher Scientific, Inc.). Hemolyzed and lipoidaemia samples were excluded. All samples were run in batches to minimize inter-assay variability and in triplicate, and the mean absorbance was calculated from the standard curve.

Statistical analysis. Statistical comparisons between groups were performed using the Student's unpaired or paired t-test. The correlation between two continuous variables was analyzed by the Spearman test. Calculations were performed using GraphPad Prism, software version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Elevated ILC2s in PBMCs are accompanied by an increased level of IL-33 in PBMCs and plasma in lung cancer patients. ILC2s, defined as lineage⁻/ICOS⁺/IL-17RB⁺ cells, were

Table I. Primers used in real-time polymerase chain reaction.

Gene	Sequence (5'-3')	Length (bp)
IL-33	Forward: ATCCCAACAGAAGGCCAAAG	198
	Reverse: CCAAAGGCAAAGCACTCCAC	
RORα	Forward: CTGACGAGGACAGGAGTAGG	204
	Reverse: GTGCGCAGACAGAGCTATTC	
GATA3	Forward: TTGTGGTGGTCTGACAGTTC	294
	Reverse: AGTACAGCTCCGGACTCTTC	
T-bet	Forward: CGGGAGAACTTTGAGTCCAT	115
	Reverse: ACTGGTTGGGTAGGAGAGGAG	
IL-13	Forward: GGCTGAGGTCTAAGCTAAGG	370
	Reverse: GACAGCTGGCATGTACTGTG	
IL-5	Forward: ACTCTCCAGTGTGCCTATTC	102
	Reverse: CTGCTGATAGCCAATGAGAC	
IL-4	Forward: GACATCTTTGCTGCCTCCA	99
	Reverse: TACTCTGGTTGGCTTCCTTCA	
Arg1	Forward: CAAGAAGAACGGAAGAATCAGC	149
	Reverse: TTGTGGTTGTCAGTGGAGTGTT	
iNOS	Forward: CTTTCCAAGACACACTTCACCA	236
	Reverse: TATCTCCTTTGTTACCGCTTCC	

IL, interleukin; RORα, retinoic acid receptor related orphan receptor α; GATA3, GATA binding protein 3; Arg1, autophagy related 1; iNOS, inducible nitric oxide.
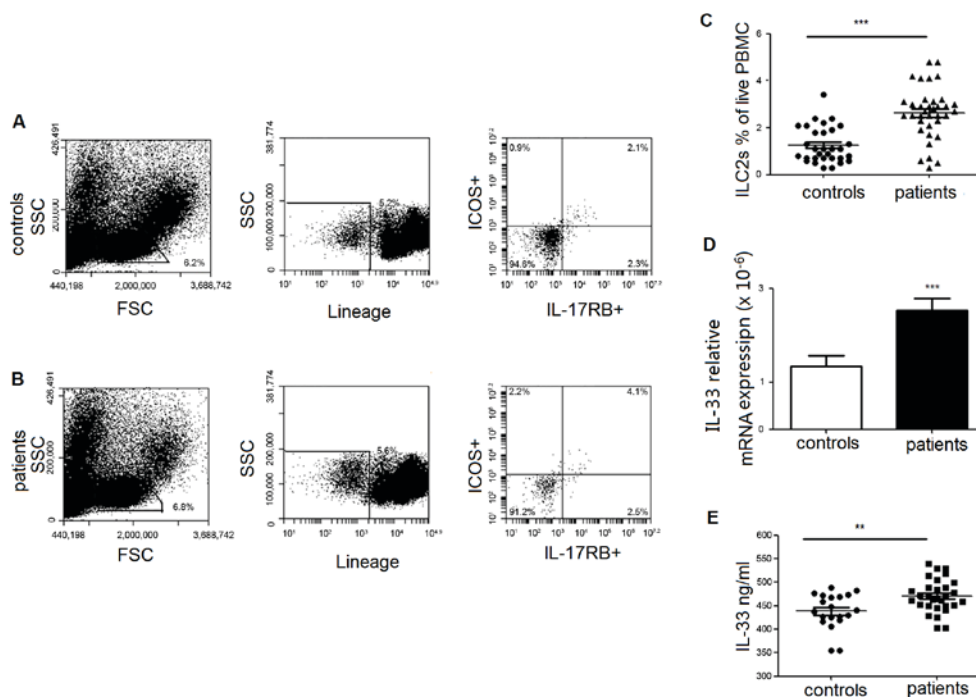

Figure 1. The results of flow cytometry analysis of ILC2s (lineage-/ICOS+/IL-17RB+) in PBMCs from lung cancer patients and healthy control. (A and B) Representative diagrams of flow cytometry analysis for circulating ILC2s. (C) The frequency of ILC2s in PBMCs from lung cancer patients was significantly increased compared with healthy controls. The IL-33 expression was clearly increased in (D) mRNA and (E) protein levels in patients with lung cancer compared with healthy controls. Data were analyzed by the Student's t-test. [***]P<0.001 and [**]P<0.01 vs. Controls. ILC2s, group 2 innate lymphoid cells; ICOS, inducible T-cell costimulator; IL-17RB interleukin (IL)-17 receptor B; PBMCs, peripheral blood mononuclear cells; SSC, side scatter characteristics; FSC, forward scatter characteristics.

determined by multicolor flow cytometry and calculated as the percentage of PBMCs. As demonstrated in Fig. 1, the frequency of individual ILC2s was significantly increased in patients compared with healthy controls. MDSCs from PBMCs of lung cancer patients were also analyzed and demonstrated a significantly elevated frequency (Fig. 2).
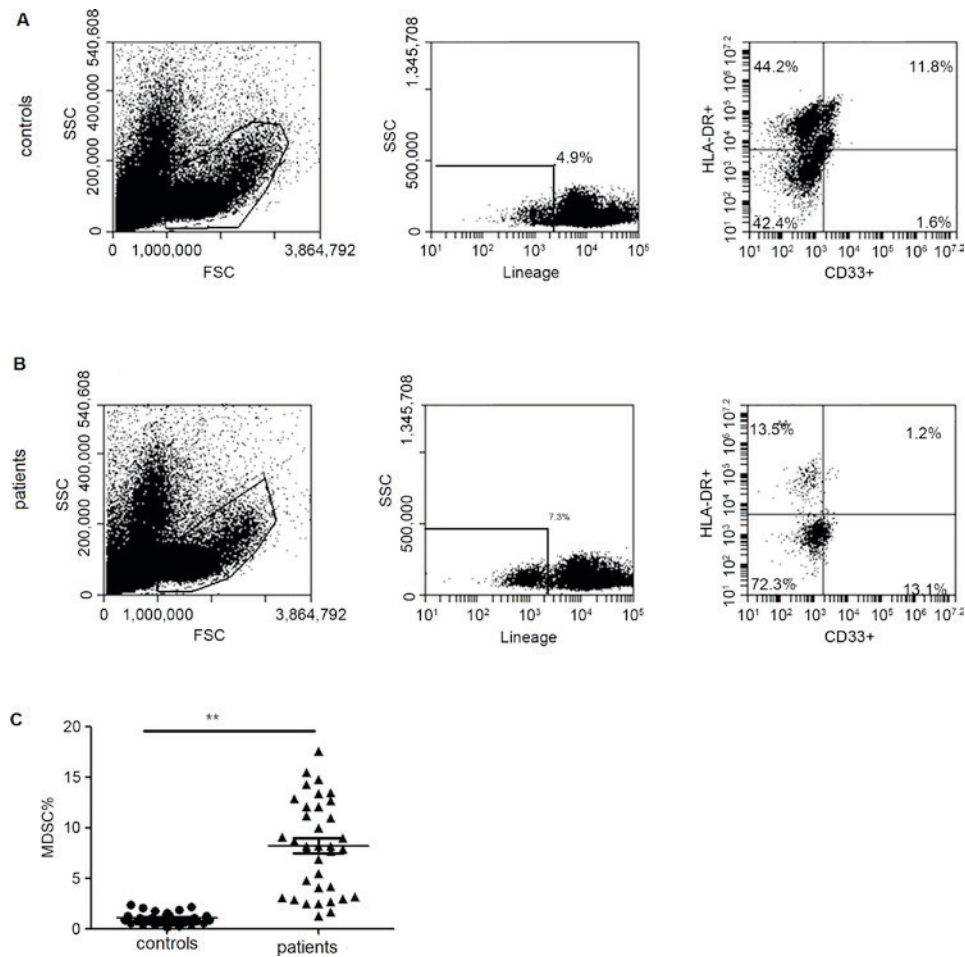
Figure 2. Elevated frequency of MDSCs (lineage-/HLA-DR⁻/CD33⁺) in PBMCs of patients. (A and B) Representative diagrams of flow cytometry analysis for circulating MDSCs. (C) The frequency of MDSCs in PBMCs from lung cancer patients, it was significantly increased compared with healthy controls. **P<0.01 vs. Controls. MDSCs, myeloid-derived suppressor cells; HLA-DR, human leukocyte antigen-antigen D; PBMCs, peripheral blood mononuclear cells SSC, side scatter characteristics; FSC, forward scatter characteristics.
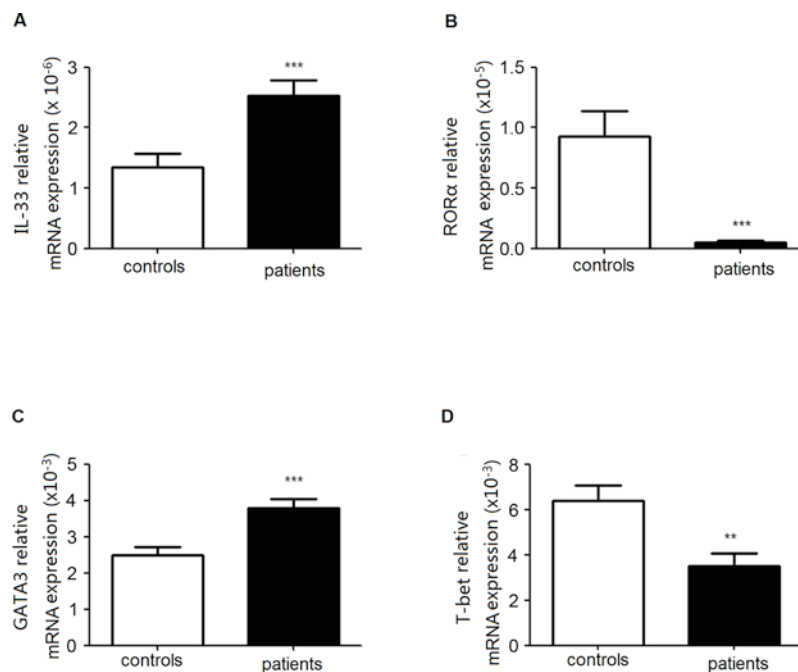

Figure 3. The levels of RORα, GATA3 and T-bet in PBMCs from patients with lung cancer. The mRNA levels of (A) IL-33, (B) RORα, (C) GATA3 and (D) T-bet was determined by RT-qPCR. Data were analyzed by the Student's t-test. All samples were measured in triplicate. **P<0.01 and ***P<0.001 vs. Controls. RORα, retinoic acid receptor related orphan receptor α; GATA3, GATA binding protein 3; PBMCs, peripheral blood mononuclear cells; IL, interleukin; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.
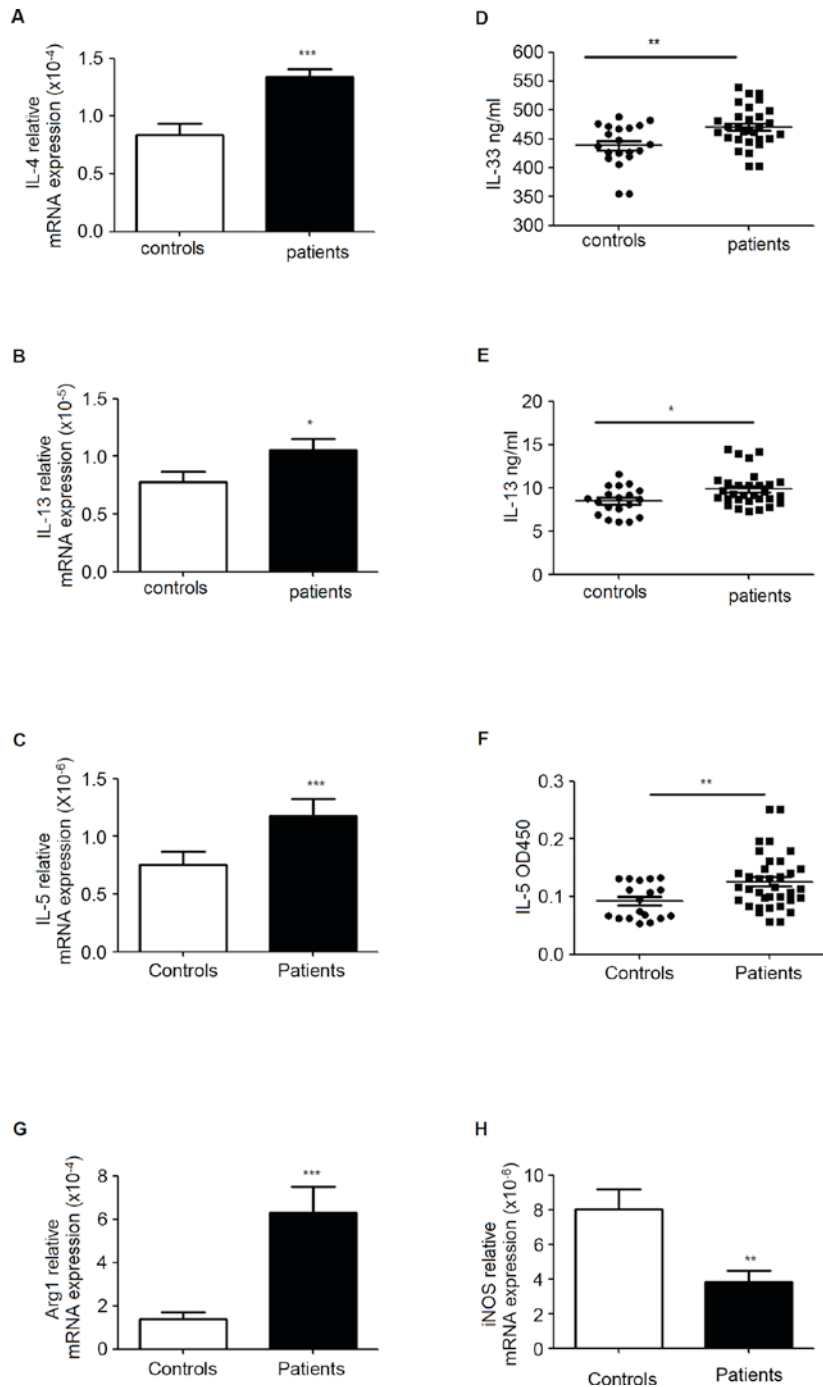
Figure 4. The levels of the ILC2s products in lung cancer. reverse transcription-quantitative polymerase chain reaction analysis of (A) IL-4, (B) IL-13 and (C) IL-5 mRNA levels in PBMCs. ELISA analysis of (D) IL-33, (E) IL-13 and (F) IL-5 protein levels in plasma. The mRNA expression levels of (G) Arg1 and (H) iNOS. Data were analyzed by the Student's t-test. All samples were measured in triplicate. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. Controls. ILC2s, group 2 innate lymphoid cells; IL, interleukin; PBMCs, peripheral blood mononuclear cells; Arg1, autophagy related 1; iNOS, inducible nitric oxide.

The plasma concentration of IL-33, an important factor with the potential to induce the dominant differentiation of human ILC2s, was also measured. The results demonstrated that IL-33 expression was clearly increased in mRNA (Fig. 1D) and protein level (Fig. 1E) in patients with lung cancer compared with healthy controls.

Increased expression levels of RORα and GATA3 in PBMCs from patients with lung cancer. The transcription factors RORα and/or GATA3 are essential for the development and function of human ILC2s. GATA3 and T-bet are involved in Th1/Th2

balance. To analyze the level of ILC2s and Th1/Th2 equilibrium in patients with lung cancer, the expression levels of RORα, GATA3 and T-bet in PBMCs were identified. As presented in Fig. 3, there was an increased mRNA expression of GATA3 in patients, although RORα mRNA decreased. Simultaneously, the expression level of T-bet was markedly decreased.

Enhanced ILC2s, MDSCs and Th2 related cytokines in PBMC from patients with lung cancer. RT-qPCR was used to analyze the mRNA levels of IL-4, IL-5, IL-13, autophagy related 1 (Arg1) and inducible nitric oxide (iNOS) in PBMCs,
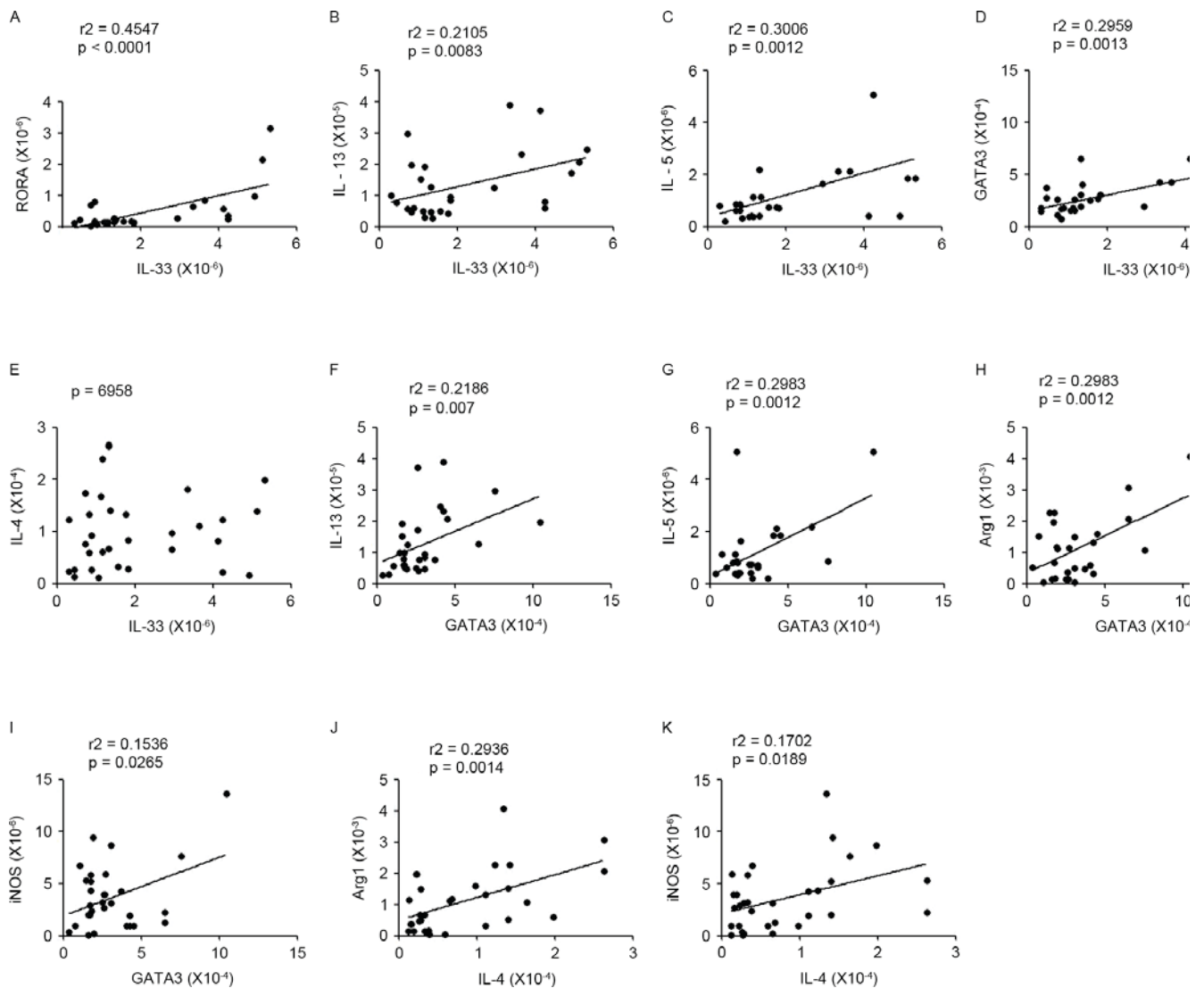
Figure 5. Correlations between Th2 cytokines and ILC2s or MDSCs. (A-C) The correlation between IL-33 and RORα, IL-5 or IL-13 expression levels. (D-G) The correlation between ILC2s associated factors (IL-33, IL-5, IL-13 or RORα) and Th2s related molecules (GATA3 or IL-4); a significantly positive correlation was observed between them in patients with lung cancer. (H-K) There were also positive correlations between GATA3/IL-4 and Arg1/iNOS mRNA expression. The data were analyzed by the Spearman test. Th2, type 2 T helper cell; ILC2s, group 2 innate lymphoid cells; MDSCs, myeloid-derived suppressor cells; IL, interleukin; RORa, retinoic acid receptor related orphan receptor α; GATA3, GATA binding protein 3; Arg1, autophagy related 1; iNOS, inducible nitric oxide.

and ELISA was performed to evaluate the levels of these signature cytokines in plasma. The data indicated that IL-5 and IL-13 were significantly increased in mRNA and protein expression levels in patients. The results also demonstrated increased mRNA levels of Arg1 and IL-4, while the level of iNOS was decreased (Fig. 4).

The correlation between the expression levels of Th2 cytokines and ILC2s or MDSCs. IL-33 serves an important role in inducing ILC2s, while IL-5 and IL-13 are the signature cytokines produced by ILC2s. Correlation analysis demonstrated that the expression of IL-33 was associated with RORA, IL-5 or IL-13 expression levels (Fig. 5A-C). In order to understand the association between ILC2s and Th2s, the connection between ILC2s associated factors (IL-33, IL-5, IL-13 or RORa) and Th2s related molecules (GATA3 or IL-4) were analyzed; the results demonstrated that there was a positive correlation between them in patients with lung cancer (Fig. 5D-G). In

addition, the mRNA expression levels of Arg1 and iNOS (the representative cytokines of MDSCs) were affected significantly and positively by predominant Th2signature cytokines in patients with lung cancer (Fig. 5H-K).

Discussion

Human immunity is an elegant, powerful and complex system that fights invading pathogens and transformed cells, including cancer. The system is divided into the major components of innate and adaptive immunity. In past years, the regulation of cancer by adaptive immunity has received a great deal of attention, although little has been focused on innate immunity. The ILC2s, which is a subset of the newly identified innate lymphoid cell family, are essential in the initiation and coordination of efficient Th2 cell-mediated immunity by producing IL-13 and IL-5 when stimulated by IL-25 or IL-33 (24). Under the tumor induced immunosuppressive environment, T helper

cells acquire Th2, while MDCS are hypothesized to inhibit effector immune responses. It has become clear that IL-4 and IL-13 are also important factors, which can promote the expansion of these MDSCs or induce their activation (10), and it has been widely accepted that the ILC2s were the early source of IL-13 (25). Therefore, it was hypothesized that these two heterogeneous groups may coexist in the tumor immune microenvironment. In the present study, the frequencies of ILC2s and MDSCs in the peripheral blood in patients with lung cancer was evaluated. It was confirmed that the circulating ILC2s (lineage$^-$/ICOS$^+$/IL-17RB$^+$) and MDSCs (lineage$^-$/HLA-DR$^-$/CD33$^+$) were significantly increased in patients with lung cancer. In addition, IL-5 and IL-13 were significantly increased in mRNA and protein expression levels in patients. It was hypothesized that the IL-13 or IL-5 produced by ILC2s may cause the expansion of circulating MDSCs or induce their activation. It was noteworthy that the expression of RORα, as a transcription factor of ILC2s, was clearly decreased in lung cancer patients in the present study. This phenomenon may be due to increased circulating MDSCs executing their inhibitory function in ILC2s by certain yet to be elucidated mechanisms, including suppression of T cell activation (16-19). A previous study (26) indicated that Th2s are also important for ILC2s function, and identified that numbers of ILC2s are not maintained following several days without T cells.

IL-33 and IL-25 are also potent activators of ILC2s, however, recent studies have paid attention on the more forcible function of IL-33 on ILC2s. Mjösberg *et al* (8) reported that human fetal gut and blood CRTH2$^+$ ILC2 lines responded to IL-25, although the response to IL-33 was more pronounced. In addition, an influenza-induced airway hyper-reactivity model confirmed the hypothesis that IL-33 is more potent in the induction of ILC2s (27-29). Consistent with these previous studies, the data from the present study demonstrated that lung cancer patients exhibited a marked increase of IL-33 in mRNA and protein levels. The mRNA level of ILC2s-required transcription factor, GATA3, was also demonstrated to be significantly increased, while the mRNA expression of transcription factor RORα was reduced compared with healthy controls. This discrepancy may due to the diversity of the tumor microenvironment, or the faster degradation rate of mRNA compared with protein (30). It has been reported (31) that GATA3 has the potential to control the function of ILC2s through multiple mechanisms. Considering the Th1/Th2 balance in this type of cancer, the mRNA level of T-bet, the Th1 cells-required transcription factor, was then analyzed in the present study and the results demonstrated that the mRNA level of T-bet was decreased, indicating that there was a predominant Th2 phenotype in patients with lung cancer.

IL-13 is one of the main products of ILC2s, and Gabitass *et al* (32) reported that the elevated MDSCs in pancreatic and gastric cancer is an independent prognostic factor associated with the elevation of IL-13. Thus, the functional products of MDSCs were analyzed; the mRNA level of Arg1 was elevated, and the level of iNOS was reduced. This phenomenon was similar to one study (33) where *in vitro* cultured MDSCs with IL-13 exhibited clearly increased Arg1, although no significantly increased iNOS. Indeed, monocytic

MDSCs appear to fit in the concept of classically activated (or M1) vs. alternatively activated (or M2) mononuclear phagocytes, induced by Th1 vs. Th2 cytokines, respectively. Monocytic MDSCs are also distinguished by the production of enzymes involved in L-arginine metabolism (high iNOS/NO for M1; high Arg1 for M2) (34). IL-4 and IL-13 are crucial for inducing the M2-MDSCs, which can produce high levels of Arg1. Studies (35-37) demonstrate that Th2s and ILC2s exhibit a close association with MDSCs. In view of this, the correlations between the products of Th2s and ILC2s or MDSCs were analyzed, and it was identified that there were positive correlations between Th2 cytokines and ILC2s or MDSCs associated factors. These results require confirmation using an *in vivo* model.

In conclusion, the present study demonstrated significant increases in circulating ILC2s and MDSCs in lung cancer patients whose peripheral circulation had an elevated expression level of IL-33. The positive correlations between Th2 cytokines and ILC2s or MDSCs associated factors indicated that enhanced ILC2s and MDSCs were accompanied by a predominant Th2 phenotype, which may lead to new immunotherapeutic approaches for lung cancer based on the associated metabolites and cytokines.

Acknowledgements

References

1. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. CA Cancer J Clin 60: 277-300, 2010.
2. Danelli L, Frossi B and Pucillo CE: Mast cell/MDSC a liaison immunosuppressive for tumor microenvironment. Oncoimmunology 4: e1001232, 2015.
3. Basith S, Manavalan B, Yoo TH, Kim SG and Choi S: Roles of Toll-like receptors in cancer: A double-edged sword for defense and offense. Arch Pharmacal Res 35: 1297-1316, 2012.
4. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, Voehringer D, Busslinger M and Diefenbach A: The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 37: 634-648, 2012.
5. Roediger B and Weninger W: Group 2 innate lymphoid cells in the regulation of immune responses. Adv Immunol 125: 111-154, 2015.
6. Halim TY, MacLaren A, Romanish MT, Gold MJ, McNagny KM and Takei F: Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. Immunity 37: 463-474, 2012.
7. Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, Camelo A, Barlow JL, Neill DR, Panova V, Koch U, *et al*: Transcription factor RORα is critical for nuocyte development. Nat Immunol 13: 229-236, 2012.
8. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, Fokkens WJ, Cupedo T and Spits H: Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat Immunol 12: 1055-1062, 2011.
9. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, Angelosanto JM, Laidlaw BJ, Yang CY, Sathaliyawala T, *et al*: Innate lymphoid cells promote lung-tissue homeostasis following infection with influenza virus. Nat Immunol 12: 1045-1054, 2011.
10. Trikha P and Carson WE III: Signaling pathways involved in MDSC regulation. Biochim Biophys Acta 1846: 55-65, 2014.

11. Mantovani A: The growing diversity and spectrum of action of myeloid-derived suppressor cells. Eur J Immunol 40: 3317-3320, 2010.
12. Khaled YS, Ammori BJ and Elkord EJ: Increased levels of granulocytic myeloid-derived suppressor cells in peripheral blood and tumour tissue of pancreatic cancer patients. Immunol Res 2014: 879897, 2014.
13. Gabrilovich DI and Nagaraj S: Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9: 162-174, 2009.
14. Feng PH, Lee KY, Chang YL, Chan YF, Kuo LW, Lin TY, Chung FT, Kuo CS, Yu CT, Lin SM, *et al*: CD14(+)S100A9(+) monocytic myeloid-derived suppressor cells and their clinical relevance in non-small cell lung cancer. Am J Respir Crit Care Med 186: 1025-1036, 2012.
15. Heuvers ME, Muskens F, Bezemer K, Lambers M, Dingemans AM, Groen HJ, Smit EF, Hoogsteden HC, Hegmans JP and Aerts JG: Arginase-1 mRNA expression correlates with myeloid-derived suppressor cell levels in peripheral blood of NSCLC patients. Lung Cancer 81: 468-474, 2013.
16. Ortiz ML, Lu L, Ramachandran I and Gabrilovich DI: Myeloid-derived suppressor cells in the development of lung cancer. Cancer Immunol Res 2: 50-58, 2014.
17. Gabrilovich D: Mechanisms and functional significance of tumor-induced dendritic-cell defects. Nat Rev Immunol 4: 941-952, 2004.
18. Bronte V, Serafini P, Apolloni E and Zanovello P: Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother 24: 431-446, 2001.
19. Sinha P, Clements VK and Ostrand-Rosenberg S: Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease. J Immunol 174: 636-645, 2005.
20. Kusmartsev SA, Li Y and Chen SH: Gr-1+ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol 165: 779-785, 2000.
21. Bie Q, Zhang P, Su Z, Zheng D, Ying X, Wu Y, Yang H, Chen D, Wang S and Xu H: Polarization of ILC2s in peripheral blood might contribute to immunosuppressive microenvironment in patients with gastric cancer. J Immunol Res 2014: 923135, 2014.
22. Xu Y, Gao J, Su Z, Dai X, Li Y, Liu Y, Chen J, Tong J, Zhang Y, Wu C, *et al*: Down regulation of Hlx closely related to the decreased expressions of T-bet and Runx3 in patients with gastric cancer may be associated with a pathological event leading to the imbalance of Th1/Th2. Clin Dev Immunol 2012: 949821, 2012.
23. Sobin LH and Compton CC: TNM seventh edition: What's new, what's changed: Communication from the International Union Against Cancer and the American Joint Committee on Cancer. Cancer 116: 5336-5339, 2010.
24. Halim TY, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, McKenzie AN and Takei F: Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity 40: 425-435, 2014.
25. Matthew T, Melissa H, Shinji T, Dawn C, Kasia G, Kelli B, Marc Q, Martin L, Tina V, Kelli B, *et al*: Respiratory syncytial virus infection activates IL-13-producing group 2 innate lyphoid cells through thymic stromal lymphopoietin. J Allergy Clin Immunol 138: 814-824.e11, 2016.
26. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, *et al*: Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 464: 1367-1370, 2010.
27. Chang YJ, Kim HY, Albacker LA, Baumgarth N, McKenzie AN, Smith DE, Dekruyff RH and Umetsu DT: Innate lymphoid cells mediate influenza-induced airway hyperreactivity independently of adaptive immunity. Nat Immunol 12: 631-638, 2011.
28. Shaw JL, Fakhri S, Citardi MJ, Porter PC, Corry DB, Kheradmand F, Liu YJ and Luong A: IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosi-nusitis with nasal polyps. Am J Respir Crit Care Med 188: 432-439, 2013.
29. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, *et al*: Innate lymphoid cells-a proposal for uniform nomenclature. Nat Rev Immunol 13: 145-149, 2013.
30. Reppert S, Boross I, Koslowski M, Türeci Ö, Koch S, Lehr HA and Finotto S: A role for T-bet-mediated tumour immune surveillance in anti-IL-17A treatment of lung cancer. Nat Commun 2: 600, 2011.
31. Wan YY: GATA3: A master of many trades in immune regulation. Cell 35: 233-242, 2014.
32. Gabitass RF, Annels NE, Stocken DD, Pandha HA and Middleton GW: Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. Cancer Immonol Immunother 60: 1419-1430, 2011.
33. Highfill SL, Rodriguez PC, Zhou Q, Goetz CA, Koehn BH, Veenstra R, Taylor PA, Panoskaltsis-Mortari A, Serody JS, Munn DH, *et al*: Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is upregulated by inter-leukin-13. Blood 116: 5738-5747, 2010.
34. Van Ginderachter JA, Beschin A, De Baetselier P and Raes G: Myeloid-derived suppressor cells in parasitic infections. Eur J Immunol 40: 2976-2985, 2010.
35. Wu Y, Yan Y, Su Z, Bie Q, Wu J, Wang S, Yu Y, Ding H, Lu P and Xu H: Enhanced circulating ILC2s accompany by upregulated MDSCs in patients with asthma. Int J Clin Exp Pathol 8: 3568-3579, 2015.
36. Klein Wolterink RG, Serafini N, van Nimwegen M, Vosshenrich CA, de Bruijn MJ, Fonseca Pereira D, Veiga Fernandes H, Hendriks RW and Di Santo JP: Essential, dose-dependent role for the transcription factor Gata3 in the development of IL-5+ and IL-13+ type 2 innate lymphoid cells. Proc Natl Acad Sci USA 110: 10240-10245, 2013.
37. Bando JK, Nussbaum JC, Liang HE and Locksley RM: Type 2 innate lymphoid cells constitutively express arginase-I in the naive and inlamed lung. J Leukoc Biol 94: 877-884, 2013.