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ORIGINAL ARTICLE



miR-192 suppresses T follicular helper cell differentiation by targeting CXCR5 in childhood asthma

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ABSTRACT

The aim of this study was to investigate the role of miR-192 in differentiation of T follicular helper cells in childhood asthma. Blood samples were taken from eighteen children with acute asthma attacks and fifteen healthy children (HC). Quantitative real-time PCR and Western blotting were used to detect the expression levels of miR-192, C-X-C chemokine receptor type 5 (CXCR5), B-cell lymphoma 6 (BCL-6) and inducible T-cell costimulator (ICOS). The flow cytometry was performed to detect the proportion of CD4 + CXCR5 + Tfh cells on CD4 + T lymphocytes. The enzyme-linked immunosorbent assay (ELISA) was carried out to determine the plasma concentrations of total IgE and IL-21. The effect of miR-192 on the T follicular helper cells differentiation by targeting CXCR5 was determined by dual-luciferase reporter assay. Children with asthma had lower levels of miR-192 than HC. The proportion of CD4 + CXCR5 + Tfh cells was significantly higher in the acute asthma group than HC. Similarly, the plasma concentration of total IgE and IL-21 in the acute group markedly increased compared with the HC, and IgE concentration was positively correlated with the proportion of CD4 + CXCR5 + Tfh cells. Furthermore, the expression levels of CXCR5, Bcl-6 and ICOS were significantly higher in the acute group than in the HC. While the proportion of CD4 + CXCR5 + Tfh cells, IL-21, CXCR5, Bcl-6 and ICOS were obviously lower in the CD4 + T cells transfected with miR-192 plasmid than that in miR-192 + CXCR5 group and control group. In conclusion, miR-192 blocks the activation pathway of Tfh cells by targeting CXCR5, which is a reasonable cellular target for therapeutic intervention.

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Childhood asthma; miR-192; CXCR5; Tfh cells; targeted modulation

Introduction

Childhood asthma is the most common disease characterized by the interactions among the genes, endogenous and exogenous factors are crucial [1,2], which results from inflammation in airways, and the effects of inflammatory cell infiltration reflect the severity of the asthma [3]. The higher antigen-specific IgE response is an identified pathogenesis of asthma [4,5]. The number of asthma patients worldwide is estimated at 334 million [6]. Meanwhile, the burden of childhood asthma is considerable in worldwide and its global prevalence has increased in the last 2–3 decades [7]. However, the pathogenesis of this disease remains poorly understood.

Follicular helper T (Tfh) cells, a specific group of differentiated CD4 + effector T cells subset located within germinal centers (GC), participate in producing antibodies from B cells [8,9]. And it has been reported that the Tfh is a subset of peripheral blood memory CD4 + T cells to regulate functional effect of memory B-cell by the change of their unique phenotype that plays a key role in development and function of Tfh and activation of cytokines [10–12]. Previous studies have observed that the peripheral Tfh compartment in

multiple autoimmune diseases was changed, such as myasthenia gravis, systemic lupus erythematosus, asthma and rheumatoid arthritis [13–16]. Some research showed that Tfh cells might contribute to the pathophysiology of asthma and correlated symptoms by the interaction among the correlative factor, such as IL-21, Bcl-6 and CXCR5, etc. [17,18].

Micro RNA (miR) is a class of small non-coding RNA chain with a regulatory function on multiple genes, immune responses and metabolic homeostasis [19]. The study had shown that change in miR-192 level was associated with asthma mechanisms, which proved the expression of miR-192 in peripheral blood and peripheral blood mononuclear cells (PBMCs) was significantly lower than that in the healthy control group [20,21]. Given this evidence of the effects of miR-192 on Tfh cells and asthma, we propose the following question: whether effects of miR-192 on asthma may not only be secondary to the effects of Tfh cells on asthma but also result from a direct target of the miR-192 to Tfh cells to influence on asthma. In this report, we showed that miR-192 exerts direct effects on CXCR5 in childhood asthma, suggesting that childhood asthma is not only a consequence of reduced Tfh cells. Furthermore, we

show that miR-192 is relevant with expression of IL-21, Bcl-6 and ICOS, which are from Tfh cells. This study showed that effects of miR-192 on differentiation of Tfh cells by targeting CXCR5 in childhood asthma.

Materials and methods

Sampling

A total of eighteen children with acute asthma (11 males and 7 females, with an average age of 3.8 ± 2.6 years) and fifteen healthy children (8 males and 7 females, with an average age of 4.6 ± 3.1 years) were selected for the study. An informed consent was signed voluntarily by all participants. Blood samples were drawn from children with acute asthma before treatment and health control (HCs) during physical examination to collect peripheral blood CD4+T cells and plasma to detect expression level of miR-192, CD4+CXCR5+Tfh cells frequencies, plasma concentration of IL-21 and IgE, respectively.

Cell sorting and transfection

Mononuclear cells (MNC) were isolated from the peripheral blood of children in our study by lymphocyte isolation liquid (Tianjin Hao Yang Biological Manufacture Co., Ltd, Tianjin, China). Circulating CD4+T cells were isolated from PBMCs using a CD4+T-cell isolation kit (Miltenyi Biotec, Bergisch, Germany) and cultured in 1640 medium supplemented with 10% serum (JRH Biosciences, Lenexa, KS) and penicillin–streptomycin liquid (Invitrogen; Thermo Fisher Scientific, MA) in a 5% CO₂ incubator at 37°C for 24 h. The miR-192 plasmid, CXCR5 plasmid, miR-192+CXCR5 plasmid and empty plasmid as control (Cyagen Biosciences Inc. Suzhou, China) were transfected into CD4+T cells using Lipofectamine 2000 according to the manufacturer specifications.

RNA isolation and real-time PCR

Total RNA from CD4+T cells and plasma was extracted with Trizol (Invitrogen, CA) and miRNeasy Serum/Plasma kit (Qiagen, Germany) according to the manufacturer's protocol. cDNA was synthesized from 1 µl RNA of plasma and cells sample with Super Script IITM reverse transcriptase with oligo (dT) and random primers (Invitrogen, CA). The primers used in the study were as follows: 5'-TTAGTTCTCAGAA TTCCAGAGGC-3' (forward) and 5'-GGACGTCTACCTCGTACAACA-3' (reverse) for BCL-6; 5'-GCCTCTGGTATTTCTTTCTCTTCTG-3' (forward) and 5'-TAGGGTCACATCTGTGAGTCTA-3' (reverse) for ICOS; 5'-CTCCTCTCCATCCACATCACCT-3 (forward) and 5'-CGTTTCTGCTTGGTTCTCTTGG-3 (reverse) for CXCR5; 5'-CAGACTAACATGCCCTTCAT-3' (forward) and 5'-GAATCTTCACTTCCGTGTGTCT-3' (reverse) for IL-21; 5'-CTGACCTATGAATTGACA-3' (forward) and reverse primer was provided in the kit for miR-192; 5'-GAAA

CGGCTTTCAGTTGAGC-3' (forward) and 5'-CTGGCCATATCCACCAGAGT-3' (reverse) for β-actin; 5'-CTCGCTTCGGCAGCACACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse) for U6. qRT-PCR was performed in a 10 µl final volume containing 0.4 µl cDNA sample, 0.2 µl primer pairs and 5 µl SYBR-Green PCR Master mix. All PCR assays were run in duplicate on an ABI PRISM 7000 (Applied Biosystems; Thermo Fisher Scientific, Inc.) system, applying 40 PCR cycles. The quantitation determined amplified PCR products using Ct values. Expression level of miR-192 was normalized against U6, and expression level of Bcl-6, CXCR5 and ICOS mRNA was normalized against β-actin, as determined by the $2^{-\Delta\Delta CT}$ method.

Flow cytometry

Density gradient centrifugation was performed to isolate peripheral blood mononuclear cells (PBMCs) as already described. After washing three times with phosphate-buffered saline (PBS), PBMCs (5×10^6 cells/100 µl) were stained with the following fluorochrome-conjugated monoclonal Abs: FITC-CD4 and PE-CXCR5 and relevant isotype controls (Biolegend, San Diego, CA) at 4°C for 30 min away from light. PBMCs were washed three times with PBS and then measured on a FACSAria II (BD Biosciences). Tfh cells were defined as CD4+CXCR5. Data were analyzed using FACSDiVa (BD Biosciences) and FlowJo 7.6 (Tree Star, Ashland, OR) software.

ELISA

Plasma levels of IL-21 and IgE were determined with ELISA (Biolegend for IL-21, BioLegend for IgE) using commercial ELISA kits according to the manufacturers' instructions. Concentrations of IL-21 and IgE were determined using standard curves of standard proteins.

Western blot analysis

The CXCR5, Bcl-6 and ICOS levels of cultured CD4+T cells and CD4+T cells transfected with miR-192 plasmid, miR-192+CXCR5 plasmid and empty plasmid were detected by western blot analysis. Different groups of cell were lysed by RIPA lysis buffer (Teknova, Hollister, CA), and BCA protein estimation kit (Beyotime, China) was used to determine the protein concentrations. Then equal protein samples were separated by 12% SDS-PAGE, transferred onto PVDF membranes. After blocked with 5% skim milk powder at room temperature for 1 h, the membranes were incubated with primary antibodies as follows: rabbit monoclonal anti-CXCR5 antibody (1:2000, Abcam), rabbit polyclonal anti-BCL-6 antibody (1:500, CST), rabbit polyclonal anti-ICOS antibody (1:1000, Abcam), rabbit monoclonal anti-GAPDH antibody (1:5000 Eptomics, U.S.A.) at 4°C overnight and secondary antibodies (Goat anti-rabbit IgG 1:10,000 H&L) at room temperature for 1 h. The results were quantified with the

Enhanced Chemiluminescence-Plus Western Blotting Detection System (Amersham, Cambridge, UK).

MicroRNA target prediction

miRNA databases and target prediction tools miRBase (<http://microrna.sanger.ac.uk/>), PicTar (<http://pictar.mdc-berlin.de/>), and TargetScan (<http://www.targetscan.org/index.html>) were used for identification of the potential targets of miR-192. We focused on targets predicted by at least two prediction databases and containing a miR-192-CXCR5 seed matching the respective 3'-UTR region.

Luciferase assay

The full length CXCR5 3'-UTR was cloned into a pGL3-Control Vector (Genechem; Shanghai, China) to create the luciferase reporter. Mutations were introduced into the corresponding position using the QuickChange Lightning Kit (Agilent Technologies, Santa Clara, CA). HEK293T cells were cotransfected with the pGL3 vectors containing the wild type or mutated 3'-UTR luciferase reporter of CXCR5 and miR-192 plasmid or the control using Lipofectamine 2000, and with Renilla luciferase (pRL-TK Vector, Promega, Madison, WI, USA) as a transfection efficiency control. After 36 h, Luciferase signal was analyzed using the Dual-Luciferase Reporter Assay kit (Promega, Madison, WI) according to the manufacturer's instructions. All experiments were repeated three times.

Statistical analyses

All the analyses were carried out by SPSS19.0 software (IBM, Armonk, NY). Differences among groups were tested by a one-way ANOVA for normally distributed data and comparison among groups by SNK test with *Kruskal-Wallis H* non-parametric test for non-normally distributed data. Statistical analyses between two groups were evaluated based on the *independent-samples T* test for normally distributed data and *Mann-Whitney U* test for non-normally distributed data. *Pearson's correlation* test for normally distributed data and *Spearman's rank correlation* test for non-normally distributed data was used to measure the possible relationship between IgE and Tfh cells count. Differences were considered statistically significant when the *p* value was under .05.

Results

miR-192 expression and frequencies of Tfh cells in childhood asthma

Previous researches have revealed that miRNAs may play a causative role in the acute asthma of experimental mouse models and chronic human asthma, for example, it has been proven the change in miR-192 expression levels may be related to asthma mechanisms [21,22]. In order to investigate the mechanism of miR-192 in the childhood asthma, we detected that expression levels of miR-192 in plasma of children with acute asthma and HC by qRT-PCR found that

miR-192 are significantly decreased compared with the control group ($p < .001$) (Figure 1(A)). Furthermore, miR-192 expression in CD4 + T cells presented a similar tendency with expression in plasma ($p < .001$) (Figure 1(B)), indicating expression level of miR-192 is closely related to childhood asthma. It was reported that the change in proportion of CD4 + CXCR5 + Tfh cells was involved in the immunological pathogenesis of children with acute asthma [23]. Our flow cytometry analysis showed that the percentage of blood CD4 + CXCR5 + Tfh cells was significantly higher in the childhood asthma than that in the HCs ($p < .001$) (Figure 1(C)). In addition, a negative correlation was found between miR-192 and frequencies of CD4 + CXCR5 + Tfh cells (Figure 1(D)). These investigations suggest that miR-192 not only down-regulated in the plasma of children with acute asthma and CD4 + T cells but also miR-192 and frequencies of CD4 + CXCR5 + Tfh cells exert negative correlation.

Alteration of concentration of IgE and the CD4 + CXCR5 + T cell-associated molecules in children with acute asthma

IgE, a marker for chronic and relapsing allergy disease, has been universally acknowledged as a risk factor for asthma onset, severity and worse prognosis in children's asthma [24,25]. We detected plasma IgE levels in all participants and analyzed its correlation with circulating CD4 + CXCR5 + Tfh cells. The results showed that plasma concentration of IgE was significantly higher in the childhood asthma patients than that in the HCs ($p < .001$) (Figure 2(A)), and a positive correlation was found between frequencies of circulating CD4 + CXCR5 + Tfh cells and plasma IgE level in all enrolled patients with acute asthma and control (Figure 2(B)).

The CD4 + CXCR5 + Tfh cell-associated molecules (CXCR5, Bcl-6, ICOS and IL-21) are crucial for the pathological progression of acute asthma in children [23]. To more fully characterize the mutual function of related molecules from CD4 + CXCR5 + Tfh cells, our subsequent analysis showed protein expression of cytokines IL-21 were significantly higher in plasma from the asthma group than the control group ($p < .001$) (Figure 2(C)). The mRNA and protein expression levels of Bcl-6, ICOS and CXCR5 from the CD4 + T cells isolated from asthma blood were significantly increased compared with the control group ($p < .001$) (Figure 2(D-G)).

CXCR5 influence on differentiation of CD4 + CXCR5 + Tfh cells by miR-192-targeting function

To further detect whether altered miR-192 and CXCR5 levels associate with asthma patient outcomes, we found CXCR5 which were significantly altered and predicted as target genes for miR-192 by databases miRanda and TargetScan. To clarify the biological mechanisms, interaction between miR-192 and 3'UTR of CXCR5 was predicted using TargetScan and Luciferase activity was assessed by luciferase

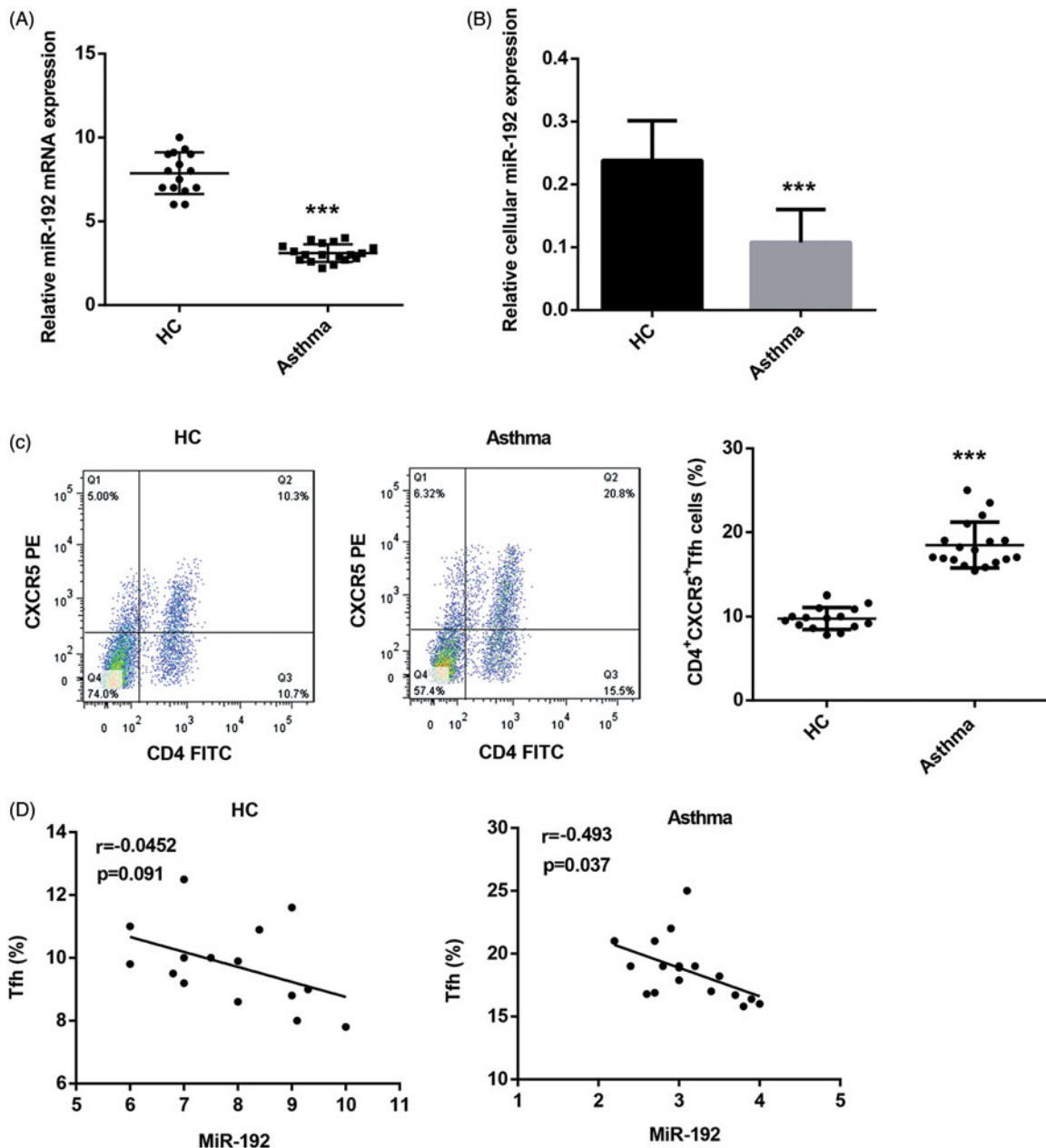


Figure 1. Expression level of miR-192 and frequency of Tfh cells in childhood asthma. (A) Comparing the levels of miR-192 in plasma from the control ($n = 15$) and asthma groups ($n = 18$). (B) Cell-specific miR-192 expression in CD4+ T cells comparing healthy control (HC) and asthmatics. (C) The ratio of CD4+CXCR5+T cells in peripheral blood from childhood with acute asthma ($n = 18$) and HC ($n = 15$) by flow cytometry, quantitative analysis of CD4+CXCR5+Tfh cells is displayed in the right. (D) Correlations between miR-192 and the frequency of CXCR5+CD4+Tfh cells. *** $p < .001$ vs. control. All data are presented as the mean $\pm$ SD.

assay (Figure 3(A)). Next, we detected miR-192 transfection efficiency (Figure 3(B)). mRNA level of Tfh cell-associated molecules (Bcl-6, ICOS and CXCR5) in CD4+ T cells transfected with miR-192 plasmid was markedly decreased (Figure 3(C–E)), and in CD4+ T cells transfected with CXCR5 plasmid was showed significantly increased than Null vector (Figure 3(C–E)). In addition, in CD4+ T cells transfected with miR-192 + CXCR5 plasmid was observably decreased compared with CXCR5 plasmid group (Figure 3(C–E)). Expressive trends of Bcl-6, ICOS, CXCR5 and IL-21 protein and frequency of CD4+CXCR5+Tfh were in consistent with mRNA levels in various comparative groups (Figures 3(F–J) and 4).

Discussion

Approximately 300 million people worldwide currently suffer from asthma, and its prevalence has increased steadily over the past few decades, particularly among children [26]. Childhood asthma is a common pediatric diseases mediated by immunoglobulin IgE [27,28]. microRNAs help control gene expression to regulate various biological processes of disease, such as proliferation, differentiation, maturation and apoptosis of several cellular [29]. Many studies have shown that the role of miRNAs in asthma, which proved profiles significantly changed in the lung tissue of the mice model mimicking and human asthma [30,31], and previous studies

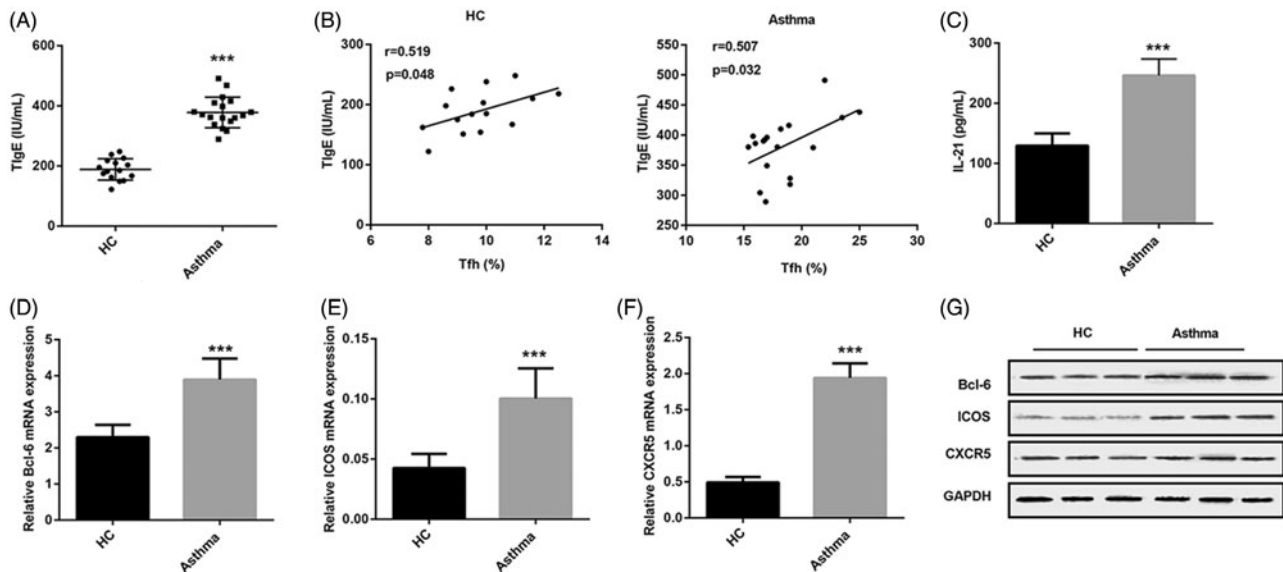


Figure 2. Alteration of Tfh cells differentiation-related molecular (A) The plasma concentration of IgE measured by ELISA. (B) Correlation between IgE and frequency of CD4 + CXCR5 + Tfh cells, IgE and CD4 + CXCR5 + Tfh cells from acute asthma and HC. (C) Comparison of plasma IL-21 level by ELISA. (D–F) Comparison of mRNA levels of BCL-6, ICOS and CXCR5 in CD4 + T cells from the control and asthma groups by qRT-PCR. (G) Western blot analysis of proteins expression levels of (BCL-6, ICOS and CXCR5) in the control and asthma groups, with β -actin as the control. Data are presented as the mean $\pm$ standard error of the mean. *** $p < .01$ vs. control.

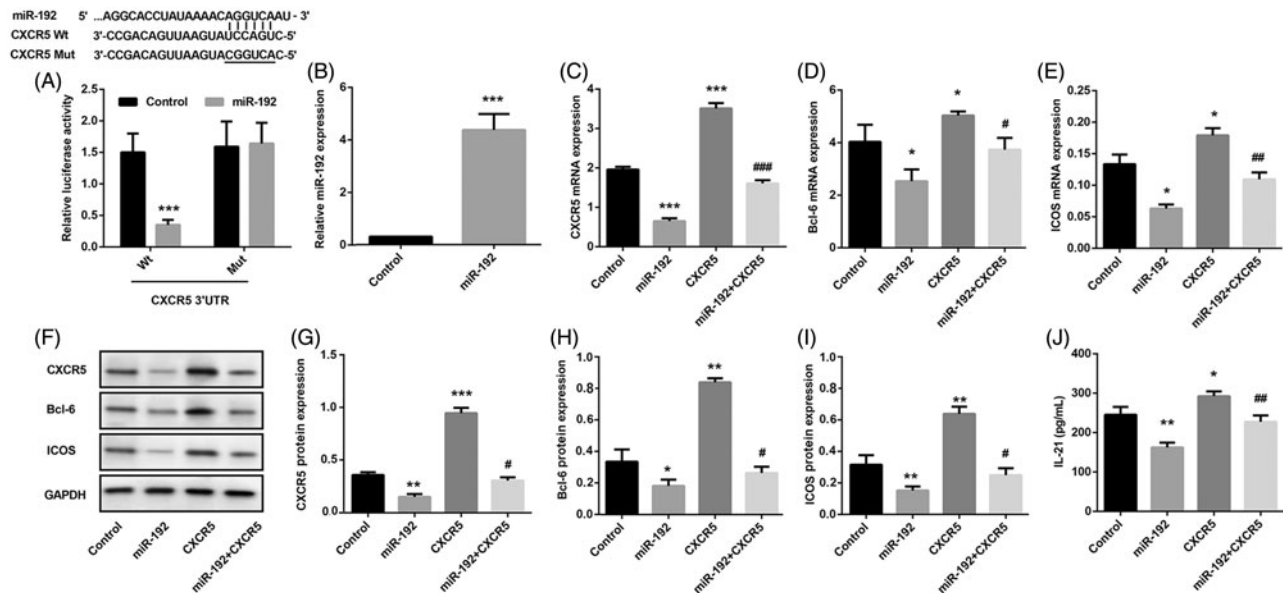


Figure 3. miR-192 suppresses T follicular helper cells differentiation by targeting CXCR5. (A) Interaction between miR-192 and 3'-UTR of CXCR5 was predicted using TargetScan and luciferase activity was assessed by luciferase assay. (B) Detection of miR-192 transfection efficiency. (C–E) Comparison of the BCL-6, ICOS and CXCR5 mRNA level. (F–I) Comparison of the BCL-6, ICOS and CXCR5 protein level. (J) Comparison of plasma IL-21 level. *** $p < .001$ vs. control, ** $p < .01$ vs. control, * $p < .05$ vs. control. # $p < .05$ vs. CXCR5 group, ## $p < .01$ vs. CXCR5 group, ### $p < .001$ vs. CXCR5 group.

have reported that miR-192 play a critical part in asthma [32]. However, the mechanism by miR-192 targeting to control the disease has remained unclear. Herein, we found that expression levels of miR-192 were significantly decreased in children with acute asthma whether in peripheral circulation or in CD4 + T cells using qRT-PCR, the results suggest that miR-192 level is closely related to children with acute asthma.

Follicular helper T (Tfh) cells, a subsets of CD4 + T cells act a critical for activation of B cells, antibody levels and GC formation, are an important factor in the pathogenesis of

allergic inflammation for Childhood asthma [33,34]. The activation of B cells normally depends on T cells, particularly Tfh cells, which located in germinal centers (GCs) [35]. Frequency of circulating CD4 + CXCR5 + Tfh cells were found in certain patients with autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis and asthma and Tfh cells is correlated with severity of disease [36,37]. High expression of chemokine (C-X-C motif) receptor 5 (CXCR5), a prominent feature of Tfh cells, can induce ICOS, BCL-6 and IL-21 to effect on phenotype and function for distinct subsets of CD4 + T cells [33,38–40].

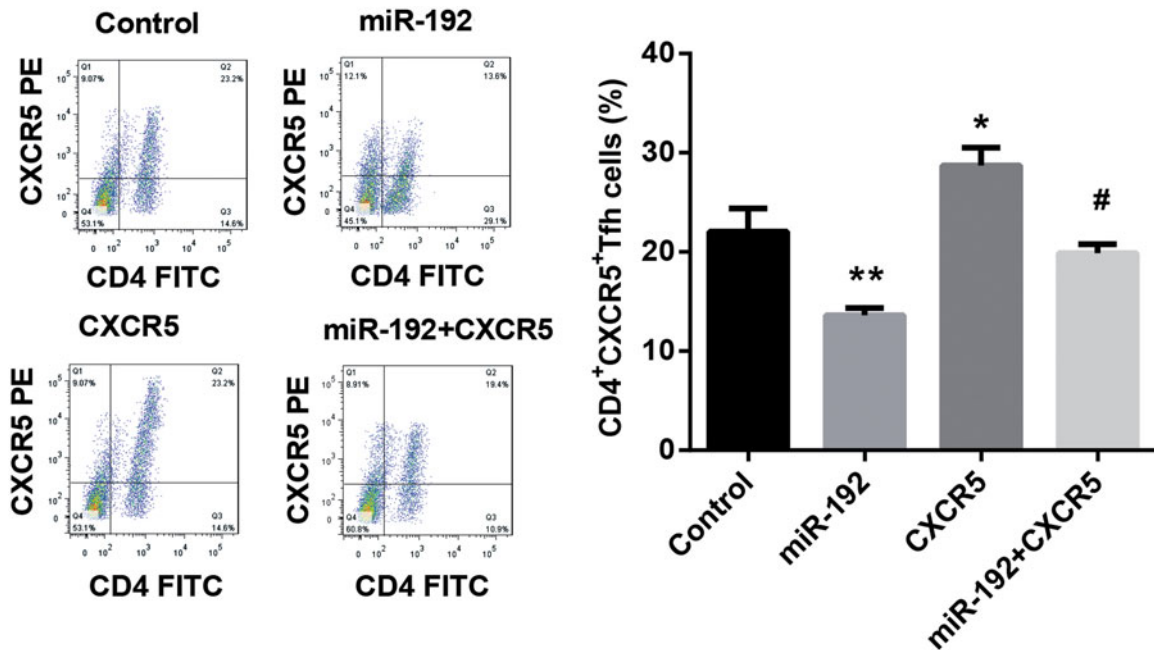


Figure 4. The frequency of circulating T follicular helper (cTfh) cells in CD4⁺T cells. All data are presented as the mean $\pm$ SD. * $p < .05$ vs. control, # $p < .05$ vs. CXCR5 group.

To investigate further the pathological process of childhood asthma, we examined the frequency of CD4⁺T cells in peripheral blood and the plasma levels of IgE and their correlation, the molecular and protein level of Tfh cells differentiation-related molecular markers including CXCR5, ICOS, BCL-6 and IL-21 and found that the percentage of blood CD4⁺CXCR5⁺Tfh cells and plasma IgE levels were significantly higher in the childhood asthma than that in the HCs, meanwhile there was a positive correlation between frequency of CD4⁺CXCR5⁺Tfh cells and plasma IgE concentrations. Taken together, these findings clarified that the elevated proportion of CD4⁺CXCR5⁺Tfh cells plays a key role in the immunological pathogenesis of acute childhood asthma. The alterations of BCL-6, plasma total IgE and cytokines IL-21 were associated with abnormality of CD4⁺CXCR5⁺Tfh cells of acute asthma in children. Combined our results with previous studies, we hypothesize that there is a certain connection between the level of miR-192 and proportion of CD4⁺CXCR5⁺Tfh cells by regulating Tfh cells differentiation-related molecular (CXCR5, ICOS, BCL-6 and IL-21).

A single miRNA can regulate a number of target genes and several miR-192 targets have been identified in different cells and organs [41]. Such as miR-192 promote cell proliferation and migration ability of NB cells by directly target Dicer1, which has already been confirmed by Galina et al. [42]. Lagos-Quintana et al and Zhang et al. indicated that miR-192 inhibits nucleotide excision repair by targeting ERCC3 and ERCC4 in HepG2.2.15 cells [43,44]. In addition, the study found that miR-192 mediates the growth-promoting effects of miR-192 in PDAC by directly target SIP1 [45]. Our previous study has showed that miR-192 level was closely associated with asthma of child. However, whether miR-192 is involved in the pathophysiological process of childhood asthma by targeting a specific gene remains

unknown. For discovering disease-specific biomarkers which take part in the pathogenesis of Childhood asthma, the bio-informatics analysis was performed to predict miR-192 target genes. Our present data suggest CXCR5 as a functional target of miR-192 by luciferase reporter gene assays, RT-PCR and Western blot analysis method, respectively. In addition, the results showed that the frequency of circulating CD4⁺CXCR5⁺T cells and its molecular markers (IL-21, BCL-6, ICOS and CXCR5) were significantly correlated with interactions between of miR-192 and CXCR5. The results illustrated that miR-192 directly combined with 3'-UTR of CXCR5 to inhibit mRNA expression to impact on disease progression.

In conclusion, our study confirmed the hypothesis that the frequency of circulating CD4⁺CXCR5⁺T cells and Allergen-specific IgE levels are altered in children with acute asthma and luciferase reporter showed that CXCR5 is the major target of miR-192 in childhood asthma.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] Duijts L. Fetal and infant origins of asthma. *Eur J Epidemiol.* 2012;27:5–14.
- [2] Trivedi MK, Sharma S, Rifas-Shiman SL, et al. Folic acid in pregnancy and childhood asthma: a US cohort. *Clin Immunol.* 2017;9922817729482.
- [3] Schmutte I, Laumonnier Y, Kohl J. Anaphylatoxins coordinate innate and adaptive immune responses in allergic asthma. *Semin Immunol.* 2013;25:2–11.
- [4] Wu LC, Scheerens H. Targeting IgE production in mice and humans. *Curr Opin Immunol.* 2014;31:8–15.
- [5] Hot topics from the Assemblies. *Breathe.* 2015;11:307–308.

- [6] Ferkol T, Schraufnagel D. The global burden of respiratory disease. *Ann Am Thorac Soc*. 2014;11:404–406.
- [7] Wong GWK, Tingfan L, Ko FWS. Changing prevalence of allergic diseases in the Asia-Pacific region. *Allergy Asthma Immunol Res*. 2013;5:251–257.
- [8] King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol*. 2008;26:741–766.
- [9] Wang Z, Ding L, Wang Z, et al. Circulating CD4(+)CXCR5(+) T cells exacerbate B cell antibody production in Crohn's disease through IL-21 secretion. *Immunol Investig*. 2015;44:665–677.
- [10] King C. A fine romance: T follicular helper cells and B cells. *Immunity*. 2011;34:827.
- [11] Xu H, Liu J, Cui X, et al. Increased frequency of circulating follicular helper T cells in lupus patients is associated with auto-antibody production in a CD40L-dependent manner. *Cell Immunol*. 2015;295:46.
- [12] Morita R, Schmitt N, Bentebibel SE, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity*. 2011;34:108–121.
- [13] Luo C, Li Y, Liu W, et al. Expansion of circulating counterparts of follicular helper T cells in patients with myasthenia gravis. *J Neuroimmunol*. 2013;256:55–61.
- [14] Simpson N, Gatenby PA, Wilson A, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum*. 2010;62:234–244.
- [15] Arroyovilla I, Bautistacaró M, Balsa A, et al. Constitutively altered frequencies of circulating follicular helper T cell counterparts and their subsets in rheumatoid arthritis. *Arthritis Res Ther*. 2014;16:500.
- [16] Gong F, Qian C, Zhu H, et al. Circulating follicular T-helper cell subset distribution in patients with asthma. *Allergy Asthma Proc*. 2016;37:154–161.
- [17] Gong F, Su Q, Jiang D, et al. High frequency of circulating follicular helper T cells in patients with bronchial asthma. *Clin Lab*. 2014;60:963–968.
- [18] Ma X, Sun Z, Zhai P, et al. Effect of follicular helper T cells on the pathogenesis of asthma. *Exp Ther Med*. 2017;14:967–972.
- [19] Fasanaro P, D'Alessandra Y, Magenta A, et al. microRNAs: promising biomarkers and therapeutic targets of acute myocardial ischemia. *Curr Vasc Pharmacol*. 2015;13:305–315.
- [20] Kam SH, Singh A, He JQ, et al. Peripheral blood gene expression changes during allergen inhalation challenge in atopic asthmatic individuals. *J Asthma*. 2012;49:219–226.
- [21] Yamamoto M, Singh A, Ruan J, et al. Decreased miR-192 expression in peripheral blood of asthmatic individuals undergoing an allergen inhalation challenge. *BMC Genomics*. 2012;13:655.
- [22] Garbacki N, Valentin ED, Huynhthu VA, et al. MicroRNAs profiling in murine models of acute and chronic asthma: a relationship with mRNAs targets. *PloS One*. 2011;6:e16509.
- [23] Cui YJ, Chen GH, Wang JL, et al. [Alterations of CD4+CXCR5+Tfh cells and its transcription regulatory factors in children with asthma]. *Zhongguo Dang Dai Er Ke Za Zhi*. 2014;16:1215–1219.
- [24] Lazic N, Roberts G, Custovic A, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy*. 2013;68:764–770.
- [25] Rø AD, Simpson MR, Storror O, et al. The predictive value of allergen skin prick tests and IgE tests at pre-school age: The PACT study. *Pediatr Allergy Immunol*. 2015;25:691–698.
- [26] Tumes DJ, Papadopoulos M, Endo Y, et al. Epigenetic regulation of T-helper cell differentiation, memory, and plasticity in allergic asthma. *Immunol Rev*. 2017;278:8–19.
- [27] O'Connell EJ. The burden of atopy and asthma in children. *Allergy*. 2004;59 Suppl 78:7–11.
- [28] Ferreira de Magalhaes MJ, Amaral RDS, Pereira AM, et al. Cost of asthma in children: a nationwide, population-based, cost-of-illness study. *Pediatr Allergy Immunol*. 2017;28:683–691.
- [29] Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;12:861–874.
- [30] Alexandrova E, Miglino N, Hashim A, et al. Small RNA profiling reveals deregulated phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K)/Akt pathway in bronchial smooth muscle cells from asthmatic patients. *J Allergy Clin Immunol*. 2016;137:58–67.
- [31] Heffler E, Allegra A, Pioggia G, et al. MicroRNAs profiling in asthma: potential biomarkers and therapeutic targets. *Am J Respir Cell Mol Biol*. 2017;57:642–650.
- [32] Yamamoto M, Singh A, Jian R, et al. Decreased miR-192 expression in peripheral blood of asthmatic individuals undergoing an allergen inhalation challenge. *BMC Genomics*. 2012;13:1–9.
- [33] He J, Tsai L, Leong YA, et al. Circulating precursor CCR7 lo PD-1 hi CXCR5+CD4+T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity*. 2013;39:770–781.
- [34] Cook KD, Shpargel KB, Starmer J, et al. T follicular helper cell-dependent clearance of a persistent virus infection requires T cell expression of the histone demethylase UTX. *Immunity*. 2015;43:703.
- [35] Craft JE. Follicular helper T cells in immunity and systemic autoimmunity. *Nat Rev Rheumatol*. 2012;8:337–347.
- [36] Zhang X, Ing S, Fraser A, Chen M, Khan O, Zakem J, Davis W, Quinet R. Follicular helper T cells: new insights into mechanisms of autoimmune diseases. *Ochsner J*. 2013;13:131.
- [37] Feng J, Lu L, Hua C, et al. Correction: high frequency of CD4+CXCR5+ TFH cells in patients with immune-active chronic hepatitis B. *PLoS One*. 2011;6:e21698.
- [38] Tangye SG, Ma CS, Brink R, et al. The good, the bad and the ugly – TFH cells in human health and disease. *Nat Rev Immunol*. 2013;13:412.
- [39] Schmitt N, Ueno H. Blood Tfh cells come with colors. *Immunity*. 2013;39:629–630.
- [40] Schmitt N, Bentebibel SE, Ueno H. Phenotype and functions of memory Tfh cells in human blood. *Trends Immunol*. 2014;35:436–442.
- [41] Lim LP, Lau NC, Garrettengele P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*. 2005;433:769.
- [42] Feinberggorenshtein G, Guedj A, Shichrur K, et al. MiR-192 directly binds and regulates Dicer1 expression in neuroblastoma. *PloS One*. 2013;8:e78713.
- [43] Zhang S, Hao J, Xie F, et al. Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development. *Carcinogenesis*. 2011;32:1183.
- [44] Lagosquintana M, Rauhut R, Meyer J, et al. New microRNAs from mouse and human. *RNA*. 2003;9:175.
- [45] Zhao C, Zhang J, Zhang S, et al. Diagnostic and biological significance of microRNA-192 in pancreatic ductal adenocarcinoma. *Oncol Rep*. 2013;30:276.