Next generation sequencing for miRNA profile of spleen CD4⁺ T cells in the murine model of acute asthma

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Aim: To explore the miRNAs profile of CD4⁺ T lymphocytes in asthma via next generation sequencing.

Methods: In the murine model of acute asthma, spleen CD4⁺ T lymphocytes were sorted, in which small RNAs were extracted and sequenced. Novel miRNAs were measured with real time quantitative reverse transcription polymerase chain reaction (qRT-PCR). **Results:** A total of 127 miRNAs were found to exhibit at least twofold change. In the 262 predicted novel miRNAs, 14 novel miRNAs were measured in qRT-PCR in the sorted CD4⁺ T cells or in the differentiated Th1/Th2 cells and novel miR-11 (xxx-m0228-3p) was significantly decreased in the sorted CD4⁺ T cells from the murine model of asthma and in the Th2 cells. **Conclusion:** Aberrant miRNAs profile in the CD4⁺ T lymphocytes from acute asthma was documented.

First draft submitted: 26 March 2018; Accepted for publication: 20 April 2018; Published online: 8 May 2018

Keywords: asthma • CD4⁺ T cells • miRNAs • next generation sequencing • Th2

Allergic asthma is a Th2-cell driven inflammatory disease [1]. Once inhaled, allergen provoked the secretion of thymic stromal lymphopoietin from bronchial epithelial cells [2]. Thereafter, allergens processed and presented by dendritic cells, with the aid of thymic stromal lymphopoietin, induce the differentiation of naive CD4⁺ T cells into Th2 cells. Th2 cells, releasing IL-4, IL-5 and IL-13, boost the immunoglobulin class switch and the production of IgE. Once bound with high affinity receptor FcεRI, IgE–allergen complex activate mast cells, which further recruit eosinophils and aggravate the disease. Th2 cells play crucial roles in the cascade reactions of allergic asthma.

miRNAs are a class of short noncoding RNAs, about 21–25 nucleotide length. Once transported into cytoplasm, miRNAs complementarily bind with 3'-untranslated regions of mRNA and inhibit the expression of target genes. Undoubtedly, miRNAs are involved with the Th2 cells polarization [3,4]. CD4⁺ T cells lacking *miR-24* and *miR-27*, which limited IL-4 production, were more prone to Th2 cell responses [5]. *miR-21* targeted *Bcl6* in the CD4⁺ T cells and increased the differentiation of Th2 cells [6]. Downregulation of *miR-31*, which targeted NF-κB-inducing kinase (*NIK*) and factor inhibiting HIF-1α (*FIH*), promoted the polarization of Th2 cells [7]. In the airway infiltrating T cells from asthmatic patients, *miR-19* targeted *PTEN* and promoted Th2 response [8]. In the mice of asthmatic model, *miR-126* blockade repressed *GATA-3* and Th2 polarization [9]. *In vitro*, *miR-155* deficiency enhanced the transcription factor c-MAF and skewed the differentiation of naive CD4⁺ T cells to Th2 cells [10]. However, in the *miR-155*^{-/-} mice, house dust mite (HDM) induced airway Th2 response was significantly diminished, suggesting that *miR-155* was required in the Th2 polarization [11].

In the murine model of asthma, miRNAs profile of lung tissues has been reported [12,13], which focused on the whole lung tissues rather than CD4⁺ T cells. Roles of miRNAs in the immune regulations have been extensively

complicated, for example, activating TLRs as single-stranded RNA molecules [14], regulating mRNAs as a decoy [15]. Despite the extensive research on miRNAs regulations on CD4⁺ T cells in respiratory diseases [16,17], direct evidences of miRNAs on CD4⁺ T cells in asthma are still limited. In the present study, we screened miRNAs profile in spleen CD4⁺ T cells from the murine model of asthma by next generation sequencing (NGS). Further, Th1 and Th2 cells were induced *in vitro*, in which some novel miRNAs were quantified with RT-PCR.

Materials & methods

Establishment of murine model of asthma

Female C57BL/6 J mice, 6–8 weeks old and free of specific pathogens, were purchased from the College of Veterinary Medicine Yangzhou University (Yangzhou, China). The animals were housed under a 12-h light/dark cycle under specific pathogen-free conditions with free access to mouse chow and water. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

The murine model of asthma was established as our previous description [24]. Mice were immunized intraperitoneally (ip.) with 20 µg of OVA (Grade V, Sigma-Aldrich, MI, USA) emulsified with 2 mg of aluminum hydroxide (InvivoGen, CA, USA) on day 1 and 15. Subsequently, the mice were boosted for 30 min via the airway with 1% OVA from day 21 to 23 consecutively. In the control group, mice were immunized and challenged with normal saline. All of the mice were monitored daily and alive before the sacrifice.

Verification of murine model of asthma

After 24 h of the last boost, C57BL/6 J mice were anesthetized by intraperitoneal injection of a mixture of 10 mg/kg xylazine (MTC Pharmaceuticals, ON, Canada) and 200 mg/kg ketamine hydrochloride (Rogar/STB, ON, Canada). The airway reactivity and dynamic compliance were as described [24]. Briefly, the mice were placed in a whole body plethysmography chamber and mechanically ventilated. The tail vein was cannulated with a 27-gauge needle for methacholine (A-2251, Sigma-Aldrich, MI, USA) administration. After the administration of increasing concentrations of methacholine, data were continuously collected from 5 s to 1 min and maximum values of airway resistance were taken to express the changes in the airway function of the mice. After the measurement of airway resistance, the sera were collected to measure total IgE using ELISA kit as instructed (Invitrogen, Thermo Fisher Scientific, MA, USA). To determine the lung tissue inflammation, the right upper lung lobe was removed, fixed, dehydrated and embedded in paraffin. The fixed embedded tissues were cut into 5-µm sections on a Leica model 2165 rotary microtome (Leica, Nussloch, Germany) and the tissue slides were stained with hematoxylin & eosin.

CD4⁺ T cells purification from mouse spleen

The mice were anesthetized with the mixture of xylazine and ketamine ip. and sacrificed. The spleen was removed, grinded and prepared into single cell suspensions. CD4⁺ T cells in spleen were sorted using CD4 (L3T4) MicroBeads (130-049-201, Miltenyibiotec, CA, USA). Briefly, the single cell suspensions were incubated with CD4 (L3T4) MicroBeads and the magnetically labelled cells were flushed and collected. Finally, the purity of CD4⁺ T cells was quantified using Anti-CD4-FITC (130-091-608, Miltenyibiotec, CA, USA).

RNA preparation & small RNAs sequencing

Total RNA from spleen CD4⁺ T cells was extracted using miRNeasy Mini Kit (QIAGEN 217004, Germany) and passed the RNA quality control for Illumina HiSeq™ 2500 sequencing. Briefly speaking, small RNAs with 18–40 nucleotides were separated with electrophoresis. After small RNAs were modified with 5' and 3' end adaptors, small RNAs were amplified and reverse transcribed into cDNAs with PCR and the cDNAs were sequenced. Clean sequenced reads (excluding reads containing ambiguous base and adaptor contaminants) were used for further analysis. BWA-backtrack comparisons for mapping small RNA sequences against mouse genome were conducted [25]. Distributions of small RNAs in genome were displayed as Circos [26,27]. Further, small RNAs were annotated with libraries for micro RNA (miRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and piwi-interacting RNA (piRNA) [28–31]. miRNAs databank was from miRBase version 21 (www.mirbase.org). Databank for rRNA, tRNA, snRNA and snoRNA was from Rfam11.0 (rfam.xfam.org) and piRNA bank was from pirnabank.ibab.ac.in. The value of miRNA was normalized with reads per million formula, which calculated the number of reads mapping to miRNAs in 1 million of clean reads [32–35].

Th1/Th2 polarization *in vitro*

Naive CD4⁺ T cells in spleen were sorted using Naive CD4⁺ T Cell Isolation Kit (130-104-453, Miltenyibiotec, CA, USA). Briefly, the single cell suspensions were incubated with biotin-antibody cocktails, which depleted the non-T cells and memory CD4⁺ T cells. The untouched naive CD4⁺ T lymphocytes were seeded into anti-CD3 (1mg/ml, 85-16-0031-85, eBioscience, CA, USA) precoated 96-well-plates. For the Th1 polarization, naive CD4⁺ T cells were further stimulated with anti-CD28 (2 µg/ml, 85-16-0281-85, eBioscience), IL-2 (20 ng/ml, 212-12, PeproTech, NJ, USA), IL-12 (50 ng/ml, 210-12, PeproTech) and anti-IL-4 (10 µg/ml, 85-16-7041-85, eBioscience). For the Th2 polarization, naive CD4⁺ T cells were further stimulated with anti-CD28 (2 µg/ml, 85-16-0281-85, eBioscience), IL-2 (20 ng/ml, 212-12, PeproTech), IL-4 (200 ng/ml, 214-14, PeproTech), anti-IFN-γ (10 µg/ml, 85-16-7311-85, eBioscience), anti-IL12 (10 µg/ml, 85-16-7123-85, eBioscience). Half of the complete RPMI-1640 medium (11875119, Gibco, Shanghai, China) with 10% FBS (Gibco, New Zealand) was replaced with fresh medium to maintain the cytokine environments. CD4⁺ T cells were harvested at day 5 for flow cytometry analysis.

Flow cytometry analysis

To measure the polarization of CD4⁺ T lymphocytes, the cells were stimulated with PMA (70-CS1001, Multiscience, China) and blocked with BFA (70-CS1002, Multiscience, China). 6 h postchallenge, the cells were harvested, gently washed in PBS containing 1% bovine sera albumin and fixed in IC Fixation Buffer (00-822-49, eBioscience). The fixed cells were permeabilized with Permeabilization Buffer (00-8333-56, eBioscience) and stained with PE-Anti-IFN-γ (85-12-7311-84, eBioscience), PE-Anti-IL4 (85-12-7041-83, eBioscience) or the isotype control. The stained CD4⁺ T lymphocytes were analyzed in BD FACS Calibur.

RNA quantification

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to confirm miRNA expression in each CD4⁺ T cells *in vivo* or *in vitro*. Briefly, miRNAs were extracted from CD4⁺ T cells using miRNeasy Mini Kit (217004, QIAGEN, USA), reversed into cDNA using Mir-X miRNA First Strand Synthesis Kit (RR820A, TAKARA, Japan), and quantified using SYBR Premix Ex Taq™ II (638515, TAKARA, Japan). The relative expression level of miRNAs was normalized to internal control U6 by using 2^{-ΔΔC_t} cycle threshold method. For T-bet, GATA-3, IFN-γ and IL-4 mRNA analysis, data were normalized by the level of β-actin expression. The primers used were shown in Supplementary Experimental Procedures.

Bioinformatics analysis

The novel miRNAs were predicted and optimized using Mireap [36,37]. IsomiRs were excluded and the second structure of novel miRNAs should be stable, with free binding energy not exceeding 20 kcal/mol. Also, novel miRNAs should be located in the intergenic regions.

miRNA differences between the control group and the murine model of asthma were calculated with edgeR, in which $p < 0.05$ was considered as significant. The threshold value for miRNA difference was the $|\log_2(\text{fold change})| \geq 1$ [38,39]. Cluster analysis was further conducted to explore the miRNAs with potential similar functions [40].

Target genes of known miRNA were predicted in the TargetScan (www.targetscan.org) [41], miRDB (<http://mirdb.org/>) [42,43] and RNA22 (<https://cm.jefferson.edu/rna22>) [44]. Venn maps were constructed to define genes that were targeted by all the three algorithms. For the novel miRNAs, the target genes were predicted in miRDB and the functions of target genes were annotated in GO Enrichment analysis (<http://geneontology.org/page/go-enrichment-analysis>) [45,46] to predict molecular functions, biological processes, cellular components, protein classes and signal pathways.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad, CA, USA). Results for variables that were normally distributed are displayed as the mean ± standard error of the mean. An ANOVA was performed to establish equal variance, and two-tailed Student's t-test with Bonferroni correction was applied to determine statistical significance, which was defined as $p < 0.05$.

Figure 1. CD4⁺ T cells purification from mice of asthmatic model. (A) In the ematoxylin & eosin staining, inflammatory infiltration in the lung was more evident in the mice challenged with OVA, bar = 20 μ m. (B) Total sera IgE was significantly increased in the mice from asthma group, * $p < 0.05$. (C) Airway resistance was significantly elevated in the mice from asthma group, * $p < 0.05$. Representative results of two independent experiments and there were five mice in each group. (D) Purity of CD4⁺ T cells sorted from spleen was almost 100%, representative result of five independent experiments.

Results

miRNAs predominated in small RNAs profile of CD4⁺ T cells

As the well-established murine model of asthma, evident pulmonary inflammation, airway hypersensitivity and higher IgE in sera were recorded in the mice with OVA challenge (Figure 1 A–C). Spleen CD4⁺ T cells were sorted and validated (Figure 1D). Further, small RNAs were extracted from and sequenced. All of the data have been submitted to Sequence Read Archive (SRA, www.ncbi.nlm.nih.gov/sra) with the accession number of PRJNA407651. In the CD4⁺ T cells from murine model of asthma, totally 1,102,216 small RNAs counts were read. Meanwhile, 1,224,517 counts for small RNAs were read in the healthy controls. Among these small RNAs, apart from small nucleolar RNA (snoRNAs, 5.59%), piwi-interacting RNA (piRNAs, 7.15%) and other (9.49%), 73.95% were miRNAs, suggesting that miRNAs may play essential roles in the CD4⁺ T cells' differentiation.

miRNA difference of CD4⁺ T cells from mouse model of asthma

From the normalized data with healthy controls, 127 miRNAs were found to exhibit at least twofold change in expression, including 39 miRNAs with $p < 0.01$, and 15 miRNAs with $p < 0.05$ (Supplementary Table 1). Compared with miRNAs in the CD4⁺ T cells from control group, 46 miRNAs were upregulated and 8 were downregulated. The top two upregulated miRNAs were *miR-295-3p* (9.75 $\uparrow$) and *miR-294-3p* (8.9 $\uparrow$); the top two downregulated miRNAs were *miR-375-3p* (7.8 $\downarrow$) and *miR-2137* (7.4 $\downarrow$). Among all of 127 miRNAs, cluster and tree-view analysis revealed that these dysregulated miRNAs could be categorized into several subpopulations (Figure 2).

Figure 2. miRNA profile of CD4⁺ T cells. (A) Volcano plots of 54 miRNAs showing a modulation with log2 fold change > 1 and a p-value < 0.05; **(B)** Heat map and hierarchical clustering in 127 modulated miRNAs in regard to the analyzed samples. There were three mice in each group.

Functional annotations of predicated targets of differently transcribed miRNAs

To explore the functions of these miRNAs, target genes were predicted using miRDB, TargetScan and RNA22. *miR-295-3p* targeted 90 genes predicated by all three programs. In contrast, *miR-294-3p* targeted 109 genes; *miR-375-3p* targeted 5 genes and *miR-2137* targeted 4 genes (Figure 3 & Supplementary Table 2).

Predication & quantification of novel miRNAs

In contrast with microarray, one advantage of NGS was the discovery of novel miRNAs. Indeed, 262 novel miRNAs were predicated in the small RNAs profiles of CD4⁺ T cells (Supplementary Table 3). Among all of these predicted miRNAs, 14 novel miRNAs were randomly selected and further quantified in qRT-PCR (Table 1). All of the 14 novel miRNAs could be successfully amplified. Of note, compared with CD4⁺ T cells from control mice,

Figure 3. Functional annotations of predicted targets of representative miRNAs using miRDB, TargetScan and RNA22. (A) miR-295-3p targeted 90 genes; (B) miR-295-3p targeted 104 genes; (C) miR-375-3p targeted 5 genes; (D) miR-2137 targeted 4 genes.

novel miR-1, miR-3 and miR-5 were increased, in which the increase of miR-3 was significant. Moreover, novel miR-11 was significantly decreased (Figure 4). *In vitro*, naive CD4⁺ T cells were induced to either Th1 cells or Th2 cells, which were hallmarked with transcription factor (T-bet, GATA-3) and cytokines (IFN- γ , IL-4). Novel *miR-1*, *miR-3*, *miR-5* and *miR-11* were quantified in Th1 and Th2 cells. In accordance with the *in vivo* observation, novel *miR-1*, *miR-3* and *miR-5* were increased in Th2 cell *in vitro*. Of note, *miR-11* was significantly decreased in the Th2 cells (Figure 5), which suggested that novel *miR-11* might play key roles in the Th2 differentiation.

Table 1. Randomly selected 14 novel miRNAs in the CD4⁺ T cells for qRT-PCR.

miRNA	Novel-id	Sequence
Novel miRNA 1	xxx-m0010-3p	AUCCAUCGGUCUGACAGACU
Novel miRNA 2	xxx-m0043-3p	CUGUGUCGGUCGGGUGGGGC
Novel miRNA 3	xxx-m0057-5p	GUCUGGACACAUGGGCUUUUU
Novel miRNA 4	xxx-m0071-5p	AGAUCAGAAGAGCACACGGC
Novel miRNA 5	xxx-m0084-5p	ACAUAACGUGGAGCUGAGUC
Novel miRNA 6	xxx-m0081-3p	UCUUCUGAAACAAUCCUUU
Novel miRNA 7	xxx-m0186-5p	CACAUCAACGUGGAGCUGAGU
Novel miRNA 8	xxx-m0304-3p	CGCCUGUCUGAGUGUGGCA
Novel miRNA 9	xxx-m0233-3p	CUGCAGGAGCUAUGAGAUGU
Novel miRNA 10	xxx-m0027-3p	UUGGUGGGUGCAUUGUUUU
Novel miRNA 11	xxx-m0228-3p	AGCAGGCGCAGGGGUGGUGGU
Novel miRNA 12	xxx-m0344-3p	CAGACCUGGAAGGCCUGGCGC
Novel miRNA 13	xxx-m0216-3p	CCUGGGCCGCGUUUCCGCG
Novel miRNA 14	xxx-m0124-5p	CCGAGGUGGGAUCCCGAGCCU

Figure 4. Novel miRNAs in the CD4⁺ T cells. (A) Heat map in 14 predicted novel miRNAs, three mice in each group. (B) qRT-PCR of above 14 novel miRNAs in CD4⁺ T cells from the murine model of asthma and the control mice. All of novel miRNAs could be successfully amplified. Novel miR-3 was significantly increased and novel miR-11 was significantly decreased, $p < 0.05$, the representative results of two independent experiments and there were five mice in each group. qRT-PCR: Quantitative real-time polymerase chain reaction.

GO enrichment analysis of novel miR-11 target genes

The target genes of novel *miR-11* were predicted in miRDB. Target rank top ten genes were listed in the Table 2. GO enrichment analysis revealed that novel *miR-11* may regulate binding and catalytic reaction in molecular function. In the biological process, *miR-11* may be involved with the cellular process and developmental process.

Figure 5. Quantification of novel *miR-1*, *miR-3*, *miR-5* and *miR-11* *in vitro*. (A & B) Transcription factor Tbet and cytokine IFN-γ were significantly elevated in Th1 lymphocytes; * $p < 0.05$. (C & D) Transcription factor GATA-3 and cytokine IL-4 were significantly elevated in Th2 lymphocytes; * $p < 0.05$. (E) Intracellular cytokine staining flow cytometry analysis of Th1 and Th2 cells. (F) Novel *miR-1*, *miR-3*, *miR-5* were increased in Th2 cell *in vitro*. Novel *miR-11* was significantly decreased in the Th2 cells * $p < 0.05$, the representative results of two independent experiments and there were five mice in each group.

Table 2. Top ten predicted targets of novel miR11.

Gene	Full name
<i>Sez6</i>	Seizure related gene 6
<i>Myh10</i>	Myosin, heavy polypeptide 10, nonmuscle
<i>Vps25</i>	Vacuolar protein sorting 25 (yeast)
<i>Hmx2</i>	H6 homeobox 2
<i>Opcml</i>	Opioid binding protein/cell adhesion molecule-like
<i>Camk2g</i>	Calcium/calmodulin-dependent protein kinase II γ
<i>Usp9x</i>	Ubiquitin specific peptidase 9, X chromosome
<i>Eif4h</i>	Eukaryotic translation initiation factor 4H
<i>Snx8</i>	Sorting nexin 8
<i>Lingo4</i>	Leucine rich repeat and Ig domain containing 4

In the cellular component, *miR-11* mainly might function in the cell part. In the protein class GO analysis, the potential function of *miR-11* may be nucleic acid binding, transcription factor and transferase. Top ten pathways for *miR-11* target genes were EGF receptor, FGF, chemokine, integrin, interleukin, ionotropic glutamate receptor, oxidative stress, PDGF, Ras and P38 MAPK (Figure 6).

Discussion

Allergic asthma is a prevailing chronic disease. Adoptive transfer of spleen OVA-primed CD4⁺ T cells has proved to be necessary and sufficient to induce the full responsiveness [18], highlighting the pivotal roles of CD4⁺ T cells in the pathogenesis of allergic asthma. In the present study, we characterized miRNAs profiles of spleen CD4⁺ T cells using NGS from murine model of asthma.

In the NGS, small RNAs (18-40 nt) were extracted, enriched and sequenced. As anticipated, 73.95% small RNAs were miRNAs. Among the miRNAs, the most significant changed miRNAs were *miR-295-3p* (9.75 $\uparrow$), *miR-294-3p* (8.9 $\uparrow$), *miR-375-3p* (7.8 $\downarrow$) and *miR-2137* (7.4 $\downarrow$). The upregulated *miR-295-3p* and *miR-294-3p* have been recorded in miRNAs database. Their functions, however, still remain elusive. In contrast to elusive *miR-294-3p* and *miR-295-3p*, downregulated *miR-375-3p* was closely associated with Th2 differentiation in the literature. In the eosinophilic esophagitis (EoE), downregulated *miR-375-3p* in epithelium contributed to the inflammation and Th2 cells infiltration [19]. IL-13, a typical Th2 cytokine, inhibited the expression of *miR-375-3p* in epithelial cells [20]. Arguably, in the murine model of asthma, Th2 cytokine IL-13 may suppress *miR-375-3p* in CD4⁺ T cells. The second downregulated miRNA in CD4⁺ T cells was *miR-2137*. In macrophages exposed to porphyromonas gingivalis infection, *miR-2137* blocking increased IL-10 [21], suggesting that *miR-2137* may regulate IL-10 and thus contribute to Th2 differentiation. Plausibly, decreased *miR-2137* may be associated with higher IL-10 in the murine model of asthma and Th2 differentiation.

Compared with traditional miRNAs array technique, one of the advantage of NGS is that novel miRNAs, which have not been annotated in miRNAs databases, could also be observed. Due to superior sensitivity, NGS could discriminate the closely related small RNAs and detect novel small RNAs. In the present study, we identified several novel miRNAs. Especially, novel *miR-11* (xxx-m0228-3p) was significantly decreased not only in spleen CD4⁺ T cells but also in the Th2 cell *in vitro*. Therefore, novel *miR-11* may play vital roles in the CD4⁺ T cells differentiation. Based on the sequence analysis in the miRBase, novel *miR-11* was similar with *miR-1188-3p*. Interestingly, novel *miR-11* potential target genes *Camk2* and *Usp9x* were involved with CD4⁺ T cells differentiation [22,23]. Further research would be warranted to explore the roles of *miR-295-3p*, *miR-294-3p*, *miR-375-3p*, *miR-2137* and novel *miR-11* (xxx-m0228-3p) in the Th2 cells differentiation and etiology of asthma.

Our preliminary study had some limitations. First, asthma was more than a Th2-mediated disease. Th17 cells, Treg cells and other CD4⁺ T cells were also involved with the asthma pathogenesis. Yet, we only focused on the Th2 cells. Second, the miRNA profile was from spleen CD4⁺ T cells rather than the lung infiltrated CD4⁺ T cells. Initially, we indeed targeted at the lung CD4⁺ T cells. We used flow cytometry to sort CD4⁺ T cells from the lung, either from the control or the murine model of asthma. We could get enough lung CD4⁺ T cells from the murine model of asthma. However, we could not acquire enough CD4⁺ T cells from the control mice. We speculated that in the control mice lung inflammation was absent and therefore CD4⁺ T cells infiltration into the lungs were limited. Finally, OVA-induced murine model of asthma is a well-established asthma model. However,

Figure 6. GO enrichment analysis of novel miR-11 target genes predicted in miRDB. (A–D) Novel miR-11 may regulate molecular function, biological process, cellular component and protein class. (E) Top 10 pathways for miR-11 target genes were EGF receptor, FGF, chemokine, integrin, interleukin, ionotropic glutamate receptor, oxidative stress, PDGF, Ras and P38 MAPK.

HDM model may be more close to the clinical situations. Moreover, human asthma is a chronic disease and our OVA-induced murine model was constructed in 24 days, imitating the initial events of the disease. Therefore, our observations should be further examined in HDM chronic asthma model and the asthma patients.

In summary, we screened the miRNAs profiles of spleen CD4⁺ T cells from murine model of asthma and quantified 14 randomly selected novel miRNAs either in the spleen CD4⁺ T cells or in the Th2 cells *in vitro*. We hypothesized that dysregulated miRNAs profiles in CD4⁺ T cells might be a hallmark of asthma and be valuable in the diagnosis of asthma. Meanwhile, further study in the aberrant miRNAs, in the other words, novel *miR-11 (xxx-m0228-3p)*, may help to understand Th2 cells differentiation and asthma pathogenesis.

Conclusion

CD4⁺ T lymphocytes play vital roles in the pathogenesis of asthma. To better under the regulation of miRNAs on CD4⁺ T cells, we sorted spleen CD4⁺ T cells from murine model of asthma. And then the miRNAs profile in the CD4⁺ T cells was evaluated via next generation sequencing. Compared with the CD4⁺ T cells in the healthy controls, CD4⁺ T lymphocytes in the asthma group manifested a quite different miRNAs profile. Hundreds of miRNAs were significantly altered. Dozens of novel miRNAs were discovered, which were further validated *in vivo* and *in vitro*. The potential functions of these miRNAs were predicated. In essence, this work may add our understanding on the CD4⁺ T lymphocytes differentiation in the pathogenesis of asthma.

Summary points

- The miRNA profile in splenic CD4⁺ T cells from OVA-immunized asthma model mice was explored by the next generation sequencing.
- A total of 127 miRNAs were found to exhibit at least twofold change.
- In the 262 predicted novel miRNAs, 14 novel miRNAs were measured in qRT-PCR in the sorted CD4⁺ T cells or in the differentiated Th1/Th2 cells.
- The novel miR-11 (xxx-m0228-3p) was significantly decreased in the sorted CD4⁺ T cells from the murine model of acute asthma and in the Th2 cells.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at:

<https://www.futuremedicine.com/doi/suppl/10.2217/epi-2018-0043>

Financial & competing interests disclosure

This work was supported by National Natural Science Foundation of China grant 81770031, Natural Science Foundation of Jiangsu Province BK20171501, the 'Six Talent Peak' Project of Jiangsu Province 2013-WSN-059, project from administration of Traditional Chinese Medicine of Jiangsu Province LZ13213 and project from Jiangsu Provincial Commission of Health and Family Planning H201501. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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