

ORIGINAL ARTICLE

MicroRNA-21 expression in neonatal blood associated with antenatal immunoglobulin E production and development of allergic rhinitis

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Summary

Background The prevalence of allergic diseases has increased in the past decades. It is unknown whether expression of certain microRNAs (miRNAs) in neonatal leucocytes is correlated to IgE production and/or allergic diseases.

Objective This study investigated the association of miRNA expression in neonatal leucocytes with cord blood IgE (CBIGe) elevation and development of allergic disease.

Methods We screened for the expression of a panel of 157 miRNAs in mononuclear leucocytes from human umbilical cord blood (CB) samples with elevated CBIGe and tracked the association of down-regulated miRNA expression to the miRNA-targeted gene expression and to children with allergic rhinitis (AR).

Results Among the initial screen of 10 CB samples with elevated CBIGe, expression of eight of the 157 miRNAs was low. Of these eight down-expressed miRNAs, three remained down-regulation in a validation with other 20 CB samples, and two of the three miRNAs, miR-21 and miR-126, were significantly lower in monocytes from AR children. Further analysis of mRNA expression of the miR-21-targeted genes identified that TGFBR2 expression on monocytes was significantly up-regulated in CB with elevated CBIGe, and in AR patients. Transfection of miR-21 precursor into monocytes from patients with AR increased miR-21 expression and decreased TGFBR2 expression.

Conclusion This study demonstrated the first in the literature that lower miR-21 expression in CB and increased TGFBR2 expression is associated with antenatal IgE production and development of AR.

Keywords allergic rhinitis, miR-21, TGFBR2

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Introduction

Allergic diseases are IgE-mediated diseases that involve an increase of IgE synthesis and IgE receptor-mediated activation [1, 2]. Higher antenatal IgE production reflected in the elevation of cord blood IgE (CBIGe), although not sensitive enough to predict the development of specific allergic diseases in childhood, is associated with the development of aeroallergen sensitization [3]. Allergic diseases are inherited diseases as children from both parents with allergic diseases had much higher rate of allergic diseases than those from non-atopic parents [4]. The prevalence of allergic diseases has dramatically increased in the past two decades [5–7], particularly in children [8, 9]. This suggests that gene–environment interaction may raise an immune program such as miRNA

expression for modulation of higher IgE production and the development of allergic diseases.

Recently, specific microRNA (miRNA) profiles were shown to influence cell differentiation and organ development [10]. miRNAs are short, non-coding RNAs with 22 or 23 nucleotides that can regulate gene expression through sequence-specific base pairing with target mRNAs. Hundreds of miRNAs have been identified in worms, flies, fish, frogs, mammals, and flowering plants using molecular cloning and bioinformatics prediction strategies [11]. Several articles demonstrated a probable link between miRNAs and cancer metastasis [12], and between miRNAs and immune responses [13, 14]. The relationships between miRNA expression and IgE production and allergic disease are largely undefined. Loffler et al. [15] reported that miR-21 is associated with STAT-3

activation during B cell proliferation and Avery *et al.* [16] showed that STAT-3 activation is involved in B cell IgE production. We hypothesized that leucocyte miRNA expression levels in neonatal stage were associated with immune reaction affecting CBIgE production and postnatal development of allergic diseases. Thus, we screened for expression of a panel of 157 miRNAs in mononuclear leucocytes (MNLs) from umbilical cord blood (CB) samples, with and without elevated CBIgE (≥ 0.5 kU/L), and validated the relationship of the neonatal down-regulation of miRNA with the CBIgE elevation, and with the development of allergic rhinitis (AR) in children.

Materials and methods

Subjects and sample collection

Umbilical CB samples were collected after informed consents were obtained from parents. The study protocol was approved by the Institution Review Board of Chang Gung Memorial Hospital, Taiwan. Elevation of CBIgE was defined by ≥ 0.5 kU/L as described previously [17–19]. Children with AR were defined as sneezing, rhinorrhea, and/or nasal obstruction together with detectable specific IgE directed against common allergens in Taiwan [20]. CB samples ($n = 30$) were collected into heparinized tubes after newborn deliveries, and separated into plasma and blood cells in a centrifuge at 1500 g for 15 min [17, 18, 21]. The plasma was subjected to detection of CBIgE, and blood cells were separated into MNLs for RNA extraction. To validate whether the innate miRNA expression was correlated to the development of AR, MNLs from children ($n = 20$; mean age: 6.03 ± 0.03 years of age) with and without AR were isolated after informed consent was obtained from parents, and subjected to RNA extraction for RT-PCR analysis of miRNA expression and miRNA-target genes expression. The samples from AR patients were not from the same individuals as the CB samples. We assessed the relationship between miR-21 expression and TGFB2 expression in newborns with elevated CBIgE, and validated the relationship in children with AR. The sample size was set at 20 for validating the association of miRNA down-expression with CBIgE elevation or with AR, based on the study power of 0.8, effect size of 0.4 and the α -level of 0.05 at two-sided null hypothesis.

Cord blood immunoglobulin E detection and RNA extraction

CB IgE levels were quantified by a sensitive, low-range IgE detection system with the automatic Pharmacia UniCAP device (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) using a fluorescence enzyme immunoassay system. Peripheral MNLs and monocytes were subjected to RNA extraction with 0.5 mL of Tri-Zol solution (Invitrogen, Carlsbad, CA, USA). After thoroughly vortexing,

0.1 mL of chloroform was added to each sample (Scharlau, SA, Barcelona, Spain) for phase separation; the upper aqueous phase was transferred to a fresh diethyl pyrocarbonate (DEPC)-treated capped centrifuge tube and the same volume of isopropanol (Merck KGaA, Darmstadt, Germany) was added for RNA precipitation at -20°C for 1 h. The RNA was harvested by centrifugation at 12 000 g for 10 min at 4°C , followed by 75% ethanol precipitation.

Screening for microRNA expression by stem-loop reverse transcription and TaqMan real-time reverse transcriptase-polymerase chain reaction

Total RNA was isolated with Tri-Zol (Invitrogen) according to the manufacturer's instructions. Stem-loop, RT-PCR analysis of miRNA expression was carried out as described previously [22]. All reagents were obtained from Applied Biosystems (Foster City, CA, USA). The Human Panel Early Access kit, containing assays for the 157 miRNAs listed in the Sanger miRBase database, was used. Briefly, 5 ng of total RNA were reversely transcribed to cDNA using stem-loop primers and the Taqman MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). miRNA expression was quantified using real-time PCR (Applied Biosystems 7700 Real-time PCR System) and the Taqman Universal PCR Master Mix. Four additional miRNA sequences from Applied Biosystems were also used for further validation: hsa-miR-20 (P/N: 30093), hsa-miR-21 (P/N: 30102), hsa-miR-96 (P/N: 30203), and hsa-miR-126 (P/N: 30023), and 5S ribosomal RNA (for normalization; P/N: 30059). The comparative threshold of PCR cycles (C_t values) was used to calculate the relative miRNA expression. The PCR reactions were run at 95°C for 15 min followed by 40 cycles of denaturing at 95°C for 10 s, and annealing/extension at 60°C for 60 s. All reactions were run in triplicate. The amount of each miRNA relative to 5S RNA was calculated using the equation $2^{-\Delta C_t}$, where $\Delta C_t = C_{t_{\text{miRNA}}} - C_{t_{5S}}$.

Real-time quantitative reverse transcriptase-polymerase chain reaction analysis of mRNA expression

MicroRNA-21 and miR-126 target gene expression was measured by real-time RT-PCR detection of hydrolysis probes (TaqMan, Applied Biosystems) using the ABI PRISM 7700 System (Applied Biosystems) as described previously [23]. Samples were tested in duplicates. The RT-PCR cycling parameters were as follows for the RT reaction: 50°C , 2 min; 60°C , 30 min; and 95°C , 5 min; this was followed by 40 cycles of PCR at 94°C for 20 s and 60°C for 1 min. The results were detected in a real-time fashion and recorded on a plot showing fluorescence vs. time. RT-PCR products were also visualized on ethidium bromide-stained 1.5% agarose (Pierce Co., Rockford, IL, USA) gels, each with a 100 bp ladder (Pharmacia Biotech, Piscataway, NJ, USA) as a reference. mRNA expression of

the 10 genes, potentially regulated by miR-21 and miR-126, as searched by the Sanger miRBase software reported by Griffiths-Jones and colleagues [24, 25], was studied. The primers and probes (all from Applied Biosystems) for detecting miR-21-targeted genes were HMGB2 (P/N: Hs00357789_g1), IL-12A (P/N: Hs99999036_m1), BTK (P/N: Hs00975865_m1), IRF3 (P/N: Hs00155574_m1), TGFBR2 (P/N: Hs00559661_m1). The primers and probes (all from Applied Biosystems) for detecting miR-126-targeted genes were CXCL12 (P/N: Hs00171022_m1), CCL20 (P/N: Hs01011368_m1), LTB (P/N: Hs00242737_m1), VCAM1 (P/N: Hs00365486_m1), and IL-21R (P/N: Hs00222310_m1). Gene expression was normalized against an 18S ribosomal RNA control (P/N: Hs99999901_s1, Applied Biosystems). The increases of mRNA expression were calculated assuming 100% efficient PCR where each C_t was normalized to 18S rRNA expression, as shown by the equation at $2^{\{[C_{t1}(\text{target}) - C_{t1}(18S)] - [C_{t2}(\text{target}) - C_{t2}(18S)]\}}$, as described previously [23]. The C_{t1} (target) and C_{t2} (target) represent the C_t values for target gene expression from patients and normal control samples, respectively. C_{t1} (18S) and C_{t2} (18S) represent the C_t values for 18S gene expression in patients and normal control samples, respectively.

Immunofluorescent analysis of TGFBR2 expression

To validate whether higher mRNA expression of TGFBR2 resulted in higher functional protein expression, we used dual immunofluorescence staining to measure protein expression on infiltrating monocytes of nasal secretions isolated from children with AR. In brief, primary antibodies with mouse anti-human CD14-rhodamine (IgG2b, BD Biosciences, Franklin Lakes, NJ, USA) and mouse anti-human TGFBR2 (IgG2b, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were incubated with MNLs at room temperature for 30 min, followed by incubation with secondary antibodies with Alexa Fluor 488-labeled goat anti-mouse IgG (Molecular Probes, Eugene, OR, USA) for 30 min at room temperature in Tris-buffered saline (TBS) containing 5% rat serum for blocking non-specific binding sites. Controls were run under the same conditions, but with individually irrelevant control antibody, followed by the same secondary antibody.

Transfection of miR-21 precursor molecules into CD14 monocytes

MicroRNA-21 precursor molecules and negative control precursor miRNAs were purchased from Ambion (Austin, TX, USA). They were transfected into CD14⁺ cells at a final concentration of 20 nmol/L using oligofectamine (Invitrogen) according to the manufacturer's instructions. CD14⁺ monocytes (purity > 95%) purified by AutoMACS bead separator (Miltenyi Biotec, Bergisch Gladbach, Germany) were transfected with 60 pmol of siRNA per 2×10^5 cells and 3 μ L of oligofectamine (Invitrogen) in serum-free

RPMI 1640 medium for 4 h, and then placed in fresh RPMI supplemented with 10% fetal bovine serum for culture. The protocol used to transfect miR-21 to CD14⁺ monocytes was optimized by an efficient transfection of 80–90% GFP fluorescence and a cell viability of more than 80% using the MTT assay. CD14⁺ cells with the transfection of miR-21 or scramble RNAs were harvested 24 h after transfection, and miR-21 and TGFBR2 expression were analysed by RT-PCR as described in the previous section.

Induction of immunoglobulin E synthesis and measurement of immunoglobulin E production

To examine the effect of TGF- β 1 on IgE production, Percoll-purified mononuclear cells (MNCs) from AR or non-rhinitis controls were plated in 24-well culture plates with and without 10 ng/mL rTGF- β 1 (R&D Systems, Minneapolis, MN, USA) in complete medium consisting of RPMI-1640 media, 10% FCS (HyClone, Logan, UT, USA), 5 mg/mL L-glutamine, 50 U/mL penicillin, and 50 μ g/mL streptomycin (Invitrogen) for 16 h. Based on preliminary experiments performed to estimate the optimal dose and time for stimulation, MNCs with and without rTGF- β 1 stimulation were further cultured at a density of 1×10^6 /mL in the presence of 10 ng/mL rIL-4 (R&D Systems) and 1 μ g/mL CD40L (R&D Systems) at 37 °C for 9 days in culture plates and changed fresh medium containing rIL-4 and CD40L every 3 days. IgE levels were quantified by Pharmacia UniCAP device as described above.

Detection of miR-21-regulated genes (TGFBR2, IL-12A, IRF3, and HMGB2) by Western blotting

Western blotting was performed using purified monocytes with and without miR-21 overexpression from AR or non-rhinitis controls. Cell lysates (10 μ g) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and transferred onto a polyvinylidene difluoride membrane (PVDF; Millipore, Bedford, MA, USA). After blocking with 1% bovine serum albumin in 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% Tween-20, protein detection was performed using the ECL system. The ECL signal was quantitated using a scanner and a densitometry program (Scion Image; Scion, Frederick, MD, USA). The membranes were incubated with polyclonal rabbit antibody against TGFBR2 (Santa Cruz Biotechnology Inc.), IRF3 (Abcam Inc., Cambridge, MA, USA), IL-12A (Santa Cruz Biotechnology Inc.), HMGB2 (Abcam Inc.) at 1:750 dilution and with anti-GAPDH antibody (Chemicon International, Temecula, CA, USA) at 1:1000 dilution as an internal control.

Data and statistical analyses

Clustering of miRNA array expression between CB with and without elevated CBIgE was done by unsupervised

hierarchical clustering using the centred correlation method in the program Cluster and TreeView obtained from the website (<http://fafner.stanford.edu/~sherlock/cluster.html#formats>) [26]. Elevated CBIgE level was determined using a cut-off value of ≥ 0.5 kU/L ($> 80\%$ percentile) as described previously [17, 18, 21]. Student's *t*-test (studies with eight or more samples) or Mann-Whitney *U*-test (studies with less than eight samples) was used to analyse miRNA expression levels between newborns with and without elevated CBIgE, and between children with and without AR. A *P*-value of < 0.05 was considered statistically significant.

Results

Screening for microRNA expression associated with cord blood immunoglobulin E elevation

To identify the miRNAs associated with CBIgE elevation, we initially screened 10 CB MNL samples for a panel of 157 miRNAs. We found that eight miRNAs were consistently down-regulated in four CB samples with elevated CBIgE in comparison with six CB samples without elevated CBIgE (Fig. 1).

Validation of microRNA expression in newborns with elevated cord blood immunoglobulin E and children with rhinitis

To validate the association of neonatal miRNA expression with CBIgE production, we subjected total RNA samples from 20 CB samples with and without elevated IgE for further validation by quantitative RT-PCR. Three miRNAs: miR-21, miR-96, and miR-126 remained significantly down-regulated in the monocytes from newborns with

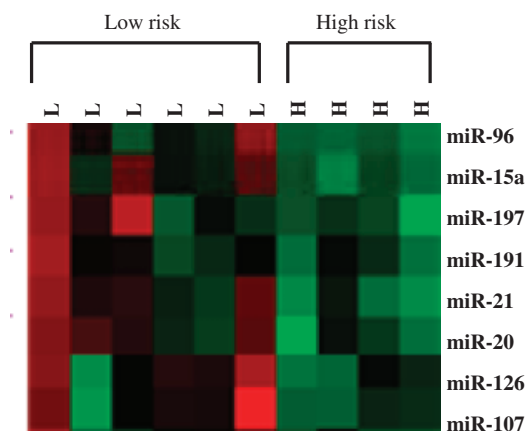


Fig. 1. Screening for microRNAs (miRNA) expression using the Human Panel Early Access kit. Total RNA samples ($n = 10$) from newborns with high risk (H) and low risk (L) to elevated cord blood IgE (CBIgE, ≥ 0.5 kU/L) were subjected to testing against the miRNAs by TaqMan real-time RT-PCR analysis. A hierarchical clustering identified the eight consistently down-regulated miRNAs shown. The green colour is for lower expression and red colour is for high expression.

elevated CBIgE (Fig. 2a). Considering that miRNAs might play a critical role during early human development and organogenesis [27–29], we investigated whether the down-regulated expression of miRNAs in the newborn stage was retained in 6-year-old children with AR. As shown in Fig. 2b, miR-21 and miR-126 remained significantly down-regulated in monocytes from children with AR in comparison with those from age-matched non-allergic children.

Correlation of the innate low miR-21, but not miR-126, expression to its targeted gene expression in newborns with elevated cord blood immunoglobulin E and children with allergic rhinitis

miRNAs act as regulators of many gene expressions. Databases for miRNA-targeted genes were developed recently; we searched an online public database system, miRBase Targets (<http://microrna.sanger.ac.uk/>) [24, 25], to validate the relationship between miR-21 expression and miR-21-targeted genes expression. We studied the

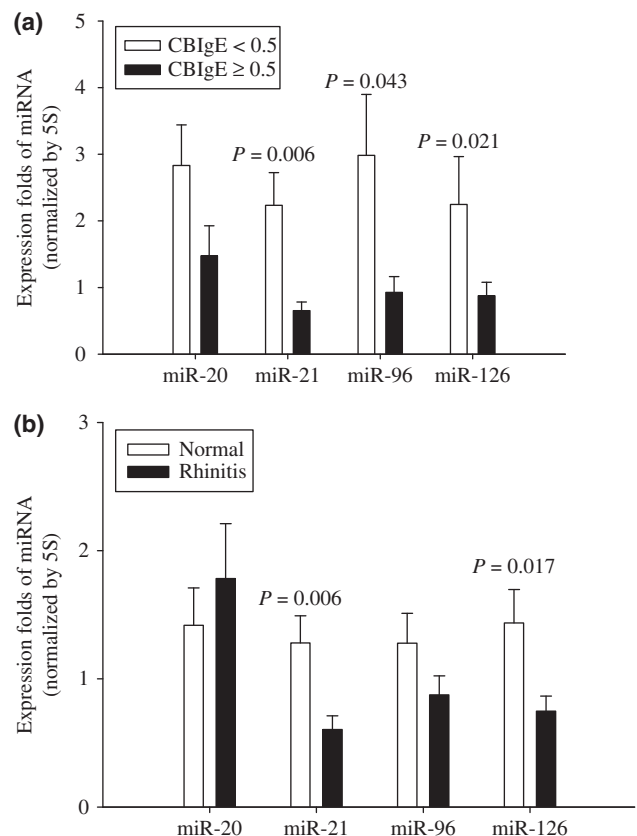


Fig. 2. Validation of down-regulation of microRNA (miRNA) expression in newborns with elevated cord blood IgE (CBIgE) and in children with allergic rhinitis. Expression of the four down-regulated miRNAs: miR-20, miR-21, miR-96 and miR-126 was validated by TaqMan real-time RT-PCR in 20 cord blood samples with and without elevated CBIgE elevation (a) and in 20 children with and without allergic rhinitis (b). Lower levels of miR-21 and miR-126 expression were significantly associated with CBIgE production and allergic rhinitis.

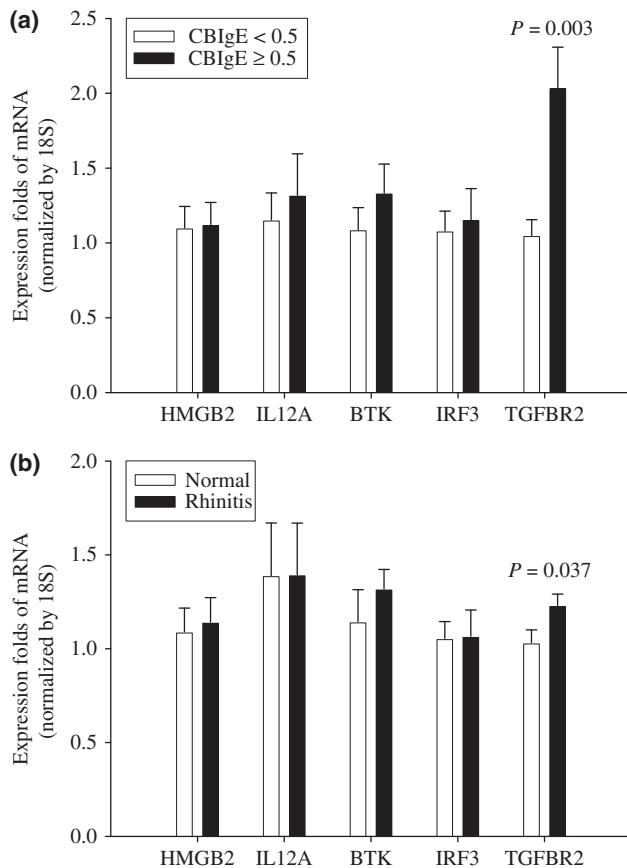


Fig. 3. Association of down-regulated miR-21 expression with its targeted genes' expression in cord blood leucocytes with and without elevated cord blood IgE (CBIgE) (a) and those from children with and without allergic rhinitis (b) by TaqMan real-time RT-PCR. TGFBR2 expression was significantly higher in newborns with elevated CBIgE and in children with rhinitis.

mRNA expression of five potentially miR-21-targeted genes among newborns with and without elevated CBIgE. As shown in Fig. 3a, TGFBR2, but not HMGB2, IL-12A, or IRF3 gene, was up-regulated and showed increased mRNA expression in monocytes from CBs with elevated CBIgE.

Further studies with monocytes from 20 AR patients vs. age-matched non-allergic children, also validated that only TGFBR2 expression was significantly increased in the children with AR (Fig. 3b). In contrast, lower neonatal expression of miR-126 was neither significantly associated with up-regulation of its target genes: LTB, IL-21R, VCAM1, CXCL12, and CCL20 mRNA expression in monocytes from newborns with elevated CBIgE (Fig. 4a), nor was it significantly associated with that from children with AR (Fig. 4b).

Increase of TGFBR2 expression on infiltrating monocytes of nasal secretion isolated from children with allergic rhinitis carrying lower miR-21 expression

Experiments were next performed to validate whether TGFBR2 expression was also present on infiltrating

CD14⁺ cells among children with AR. Higher TGFBR2 expression on infiltrating monocytes isolated from children with AR was found by immunofluorescence staining in comparison with age-matched controls (Figs 5a and b). Using flow cytometric analysis of cell surface markers, we also found that TGFBR2 expression on infiltrating monocytes was significantly higher in children with AR ($P = 0.033$; Fig. 5c).

Overexpression of miR-21 expression by precursor transfection decreased cell surface TGFBR2 expression on monocytes

To validate the cause-effect relationship between miR-21 and TGFBR2 expression in monocytes from children with AR, we transfected a double-stranded miR-21 precursor to purified CD14⁺ cells from subjects with AR. This miR-21 transfection caused significantly higher expression of miR-21 as detected by RT-PCR (Fig. 6a). The overexpression of miR-21 precursor was associated with significantly reduced TGFBR2 but not IL-12A mRNA expression

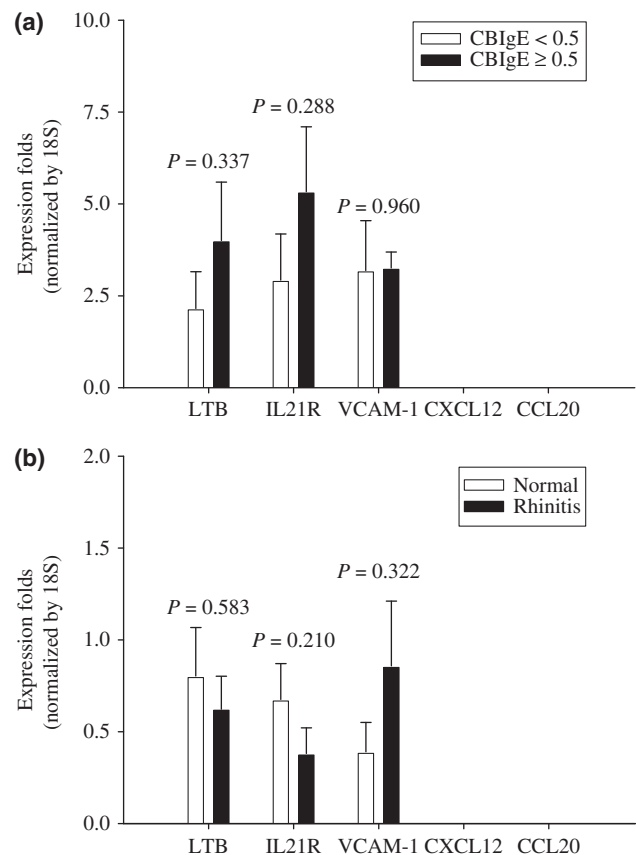


Fig. 4. Association between down-regulated miR-126 expression and its targeted genes' expression in cord blood (CB) with and without cord blood IgE (CBIgE) elevation (a) and between children with and without allergic rhinitis (b) by TaqMan real-time RT-PCR. Lower miR-126 expression in newborns with elevated CBIgE or children with rhinitis was not associated with its targeted genes' expression ($P > 0.05$).

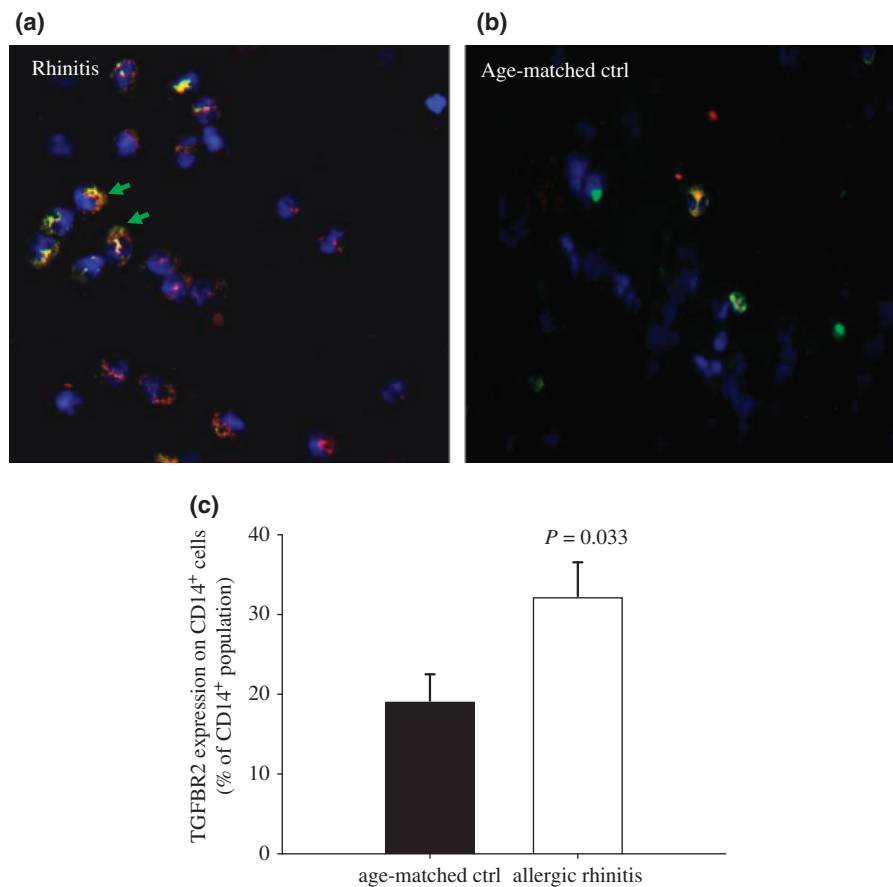


Fig. 5. Higher TGFBR2 expression on infiltrating monocytes of nasal secretions isolated from children with allergic rhinitis and low miR-21 expression. TGFBR2 expression of infiltrating monocytes from allergic rhinitis patients (a), and age-matched controls (b) was demonstrated by triple immunofluorescence staining with anti-TGFBR2 (FITC, green) and anti-CD14 (rhodamine, red) and with DAPI (blue) to visualize cell nuclei. (c) TGFBR2 expression of nasal secretion monocytes from allergic rhinitis patients and age-match controls was calculated and summarized from eight replicate experiments.

compared with the scrambled negative control experiments, as shown in Fig. 6b. The other two miR-21-targeted genes: IRF3 and HMGB2, expression revealed undetectable in the real-time RT-PCR detection. In protein expression, transfection of miR-21 precursor down-regulated TGFBR2 expression, but not other miR-21-targeted proteins: IL-12A, HMGB2 or IRF3 protein expression in monocytes by Western blotting (Fig. 6c).

To clarify whether TGFBR pathway was directly or indirectly involved in the B cell-mediated IgE production, we examined the effect of exogenous TGF- β 1 on *in vitro* IgE production by MNCs. MNCs from AR and non-rhinitis children were stimulated with rIL-4 and CD40L for 9 days in the presence of TGF- β 1 (10 ng/mL). As shown in Fig. 7, MNCs from patients with AR produced a significantly higher IgE production ($P=0.002$). Addition of TGF- β 1 suppressed IgE production by MNCs, especially in AR group ($P=0.006$). This suggests that a higher TGFBR2 expression on monocytes in AR patients might be a compensatory mechanism to suppress IgE production.

Discussion

Elevation of total IgE levels in blood has long been linked to allergic sensitization and allergic disease [30, 31]. There are at least 83 genes in 20 chromosomes associated with IgE production or allergic disorders [32–36]. This implies that allergic disease is a complex disease. There are very few studies exploring whether miRNAs are involved in IgE production early in fetal life and/or whether they are involved in postnatal development of allergic diseases. Postnatal tracking of immune parameters in children who do, vs. who do not, develop AR would provide additional information relating to the immunologic basis for the susceptibility to AR. We reported here that lower miR-21 expression in CB leucocytes was associated with high TGFBR2 expression and AR. The miR-21 involvement of TGFBR2 expression was validated by transfection of miR-21 precursor into CD14⁺ monocytes. Whether lower miR-21 and/or higher TGFBR2 expression can predict AR requires further studies in a larger cohort study.

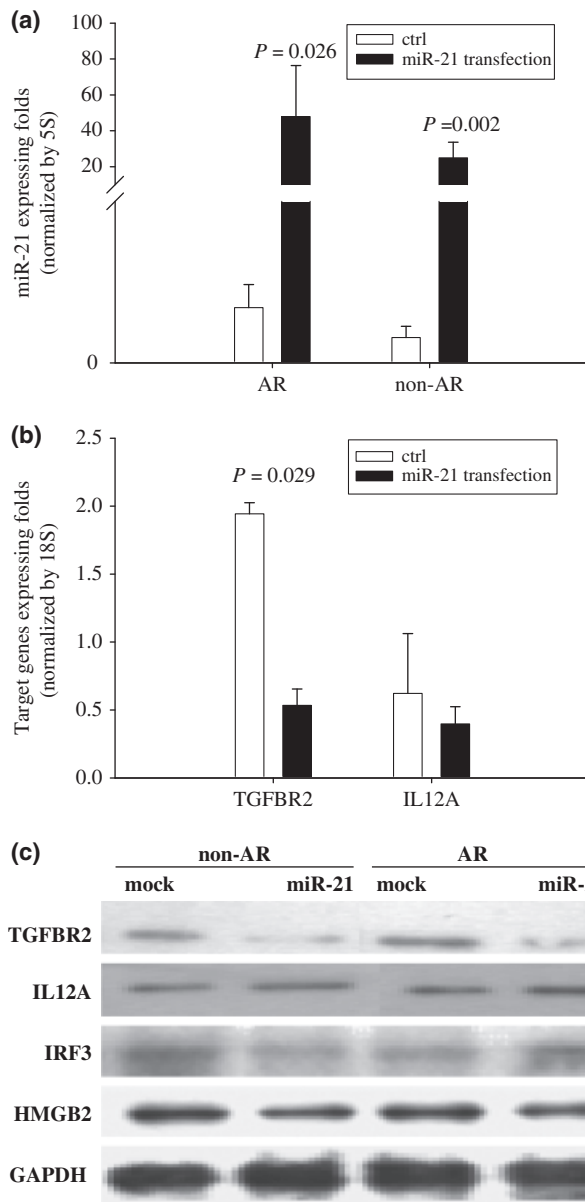


Fig. 6. Overexpression of miR-21 by precursor transfection decreased cell surface TGFBR2 expression on monocytes as detected by TaqMan real-time RT-PCR. CD14 monocytes isolated from patients with and without allergic rhinitis (AR) were subjected to miR-21 precursor transfection by oligofetamine. After miR-21 transfection for 24 h, CD14 cells expressed significantly higher miR-21 expression in children with AR (a). The up-regulation of miR-21 expression in children with AR was significantly associated with increased TGFBR2 mRNA expression, but not IL-12A mRNA expression (b). Data presented were calculated from three duplicate experiments and tested for statistical significance using the Mann-Whitney *U*-test. In Western blot analysis, it was found that transfection of miR-21 precursor downregulated TGFBR2, but not IL-12A, IRF3 or HMGB2 protein expression in monocytes by Western blotting (c).

Many miRNAs are associated with regulation of haematopoiesis [37], cancer progression [38, 39], and immune responses [40, 41]. The biological effects of miRNAs are

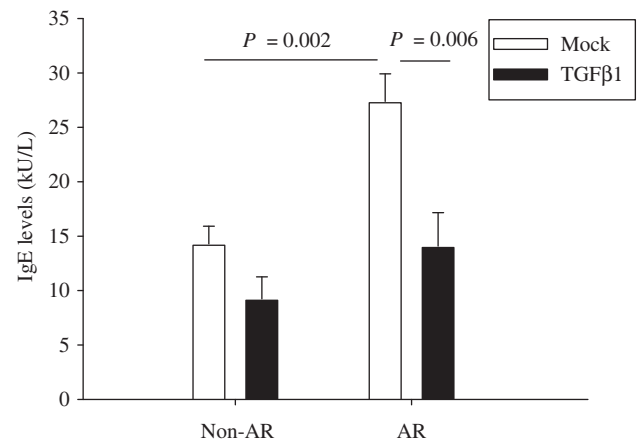


Fig. 7. TGF- β 1 suppression of the IgE production by mononuclear cells (MNCs) from allergic rhinitis (AR) and non-rhinitis patients. MNCs (1×10^6 cells/mL) were stimulated with 10 ng/mL rIL-4 and 1 μ g/mL CD40L in the presence of TGF- β 1 (10 ng/mL) for 9 days before the harvest of supernatants for measurement of IgE production. The IgE production by MNCs from AR patients was significantly higher than that from non-rhinitis patients ($P = 0.002$); presence of TGF- β suppressed the IgE production significantly in the AR patients ($P = 0.006$) but not non-rhinitis patients ($P > 0.05$). Data presented are calculated from three replicated studies.

complicated by the fact that multiple mRNAs are usually targeted by a single miRNA. MicroRNA-21 is one of the most prominent miRNAs implicated in the genesis and progression of human cancer [38], particularly in tumour growth [38], proliferation [42], and anti-apoptosis [43]. Loffler et al. [15] demonstrated that miR-21 induction by IL-6 was strictly Stat-3 dependent and Avery et al. [16] showed that Stat-3 activation was involved in IL-21-induced IgE secretion by CD40L-stimulated human naive B cells. Recently, Lu et al. [44] reported that miR-21 was up-regulated in allergic airway inflammation and down-regulated IL-12A expression in a mouse model. In contrast, we found that overexpression of miR-21 precursor in human CD14 monocytes reduced TGFBR2, but not IL-12A expression. Immune functions of different miR-21 expression in different species and/or tissues require further clarification before the potentially translational implication and application.

Three distinct cell-surface receptors for TGF- β , called TGFBR1, TGFBR2, and TGFBR3, are identified on most types of cells. Among them, TGFBR1 and TGFBR2 are important for the TGF- β functions, as indicated by studies on normal adult human skin fibroblasts [45]. Expression of mast cell TGF- β receptors was investigated in tissue sections from biopsy specimens obtained from patients with AR by Salib et al. [46]. They found a significant increase in epithelial immunoreactivity to TGFBR2 and TGFBR3 in patients with perennial and seasonal AR compared with that in control subjects without AR [46]. We have also shown that monocytes from AR patients had

higher TGFBR2 expression (Fig. 5). Thus, TGF- β receptor expression might be an effector molecule for the pathogenesis of rhinitis. However, our study (Fig. 7) and others [47] have shown that increase of exogenous and endogenous TGF- β 1 suppressed IgE production. Taken together, this suggests that the role of miR-21 in AR is complex. miR-21 may not act through B cell-mediated IgE production, but other mechanisms, such as mast cell activation to affect allergic inflammation, because augmented TGFBRs expression has been shown on mast cells from patients with AR [46]. Moreover, we did not study the miR-21 and/or TGFBR2 effect on leucocyte responses in patients with non-AR in the present study. Further studies on the tissue- and cell-specific effects of miR-21 and TGFBR2 in allergic and non-AR patients are needed before the potential rescue of allergic and/or non-AR by decreasing TGFBR2 expression or increasing miR-21 expression.

In summary, we showed that low-neonatal miR-21 expression on leucocytes was associated with high TGFBR2 expression in newborns with elevated CB IgE and in children with AR. Direct transfection of miR-21 precursor caused ectopic miR-21 expression, associated with down-regulation of TGFBR2 on CD14⁺ cells isolated from patients with rhinitis, suggesting miR-21 and TGFBR2 expression are early predictors of AR and possible therapeutic targets for the treatment of AR.

Acknowledgements

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Conflicts of interest: We declare that we have no conflicts of interest.

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