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ORIGINAL ARTICLE

Evaluation of the Possible Role of miRNAs in Chemical Allergen Potency

miRNA'ların Kimyasal Alerjen Potansiyellerinin Olası Rollerinin Değerlendirilmesi

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ABSTRACT

Objectives: MicroRNAs (miRNAs) are short, endogenous noncoding RNA molecules that can bind to certain parts of target mRNAs, thereby regulating gene expression. Studies showed that miRNAs could be up- or downregulated in different allergic skin conditions but there is still need for further studies. The aim of this study was to investigate the expression of miRNAs in response to the common contact allergen Bandrowski's base (BB), the principal allergen in patients reacting to p-phenylenediamine (PPD).

Materials and Methods: The human promyelocytic cell line THP-1 was exposed to BB at a concentration of 1 µg/mL for 24, 48, and 72 h. The dose was selected from the results of cytotoxicity assays. RNA was purified and miRNA expression profile and real-time polymerase chain reaction (RT-PCR) were performed to identify up- or downregulated miRNAs and confirm their modulations.

Results: Among the different modulated miRNAs, the upregulation of miRNA-155 and the downregulation of miRNA-21 were found to be important because these are related to immune system. This expression profile of miRNAs was also confirmed by RT-PCR.

Conclusion: These preliminary results showed that miR-155 and miR-21 may play a role in the pathogenesis of allergic contact dermatitis, but further studies are needed to clarify their definite roles.

Key words: MicroRNA, miRNA, immune system, allergic contact dermatitis, skin

ÖZ

Amaç: MikroRNA'lar (miRNA) kısa, endojen ve kodlamayan RNA molekülleri olup hedef mRNA'ların belirli bölgelerine bağlanarak gen ekspresyonunu düzenlerler. Çalışmalar, farklı alerjik deri hastalıklarında miRNA'ların arttığını ya da azaldığını göstermiştir; fakat hala daha ileri çalışmalara ihtiyaç vardır. Bu çalışmada, p-fenilendiamine reaksiyon veren hastalarda sıklıkla rastlana bir temas alerjeni madde olan Bandrowski bazına (BB) yanıt olan miRNA'ların ekspresyon profillerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: İnsan promiyelositik hücreleri (THP-1) BB'ye 1 µg/mL kosantrasyonda 24, 48 ve 72 saat maruz bırakılmıştır. Bu doz sitotoksisite deneylerinin sonuçlarına göre seçilmiştir. RNA saflaştırılmış ve miRNA ekspresyon profili ve gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) ile artan veya azalan miRNA'ları belirlemek için kullanılmıştır ve sonrasında modülasyonları doğrulanmıştır.

Bulgular: Farklı modüle edilen miRNA'lar arasında, immün sistem ile ilişkili oldukları için, miRNA-155'in düzeyinin artması ve miRNA-21'in ise düzeyinin azalması önemli bulunmuştur. Bu miRNA'ların ekspresyon profilleri aynı zamanda RT-PCR ile doğrulanmıştır.

Sonuç: Bu ön sonuçlar, miR-155 ve miR-21'in alerjik kontakt dermatit patogenezinde rolü olabileceğini göstermiştir. Ancak, kesin rollerinin açıklanabilmesi için ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: MikroRNA, miRNA, immün sistem, alerjik kontakt dermatit, deri

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INTRODUCTION

Immunotoxic agents are xenobiotics that can initiate or exacerbate the adverse immune responses in genetically susceptible persons. Drugs and various chemicals can be classified as immunotoxic compounds.[1] When low molecular weight chemicals come into contact with the skin it can lead to contact allergy and can cause allergic contact dermatitis (ACD) if exposure exceeds the personal threshold. ACD is a more common form of immunotoxic reaction in industrialized countries.[2] There are more than 4000 chemicals linked to contact allergy and ACD in humans. ACD can be prevented by proper hazard identification and labeling, by characterization of potency, by investigation of human skin exposure, and by the implementation of proper risk assessment and management strategies.[3]

P-phenylenediamine (PPD) is used commonly in dyeing hair but it is a potential skin allergen. It is found that the mechanism of reaction to PPD is linked to its oxidation products and/or metabolites. Bandrowski's base (BB) (Figure 1), 1,4-benzoquinone, is a trimer that forms quickly upon storage of PPD and it has been suggested as the principal allergen in patients reacting to PPD.[4]

THP-1 is a human leukemia promyelocytic cell line and it has been widely used in immunotoxicology studies that investigated monocyte/macrophage functions, mechanisms, and signaling pathways. This cell line has become a suitable model to estimate the modulation of monocyte and macrophage activities and it is very well suited for *in vitro* studies of chemical allergens.[5-7]

miRNAs are a class of evolutionarily conserved, single-stranded, noncoding RNA molecules including 19-24 nucleotides that play an important role in various biological processes via regulating gene expression through affecting the transcriptional and translation processes.[8] miRNAs have also been implicated in several inflammatory and immunological disorders as well as cancer.[9] It was demonstrated that miR-21 and miR-155 play a significant role in the development of the immune system. miR-21 controls the apoptosis of immune cells and miR-155 is an important factor controlling lymphocyte differentiation and functions.[10,11] Analysis of microRNAs (miRNAs) has powerful potential for the identification of novel prognostic or predictive biomarkers. Although several studies have evaluated the impact of miRNAs on immunotoxic processes and allergic skin conditions, these studies are not sufficient to allow a conclusion to be drawn.[12] Therefore, the aim of the present study was to evaluate miRNA profiles that might play a role in chemical allergen potency.

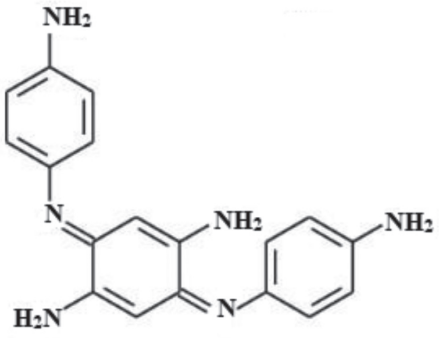

Figure 1. Chemical structure of Bandrowski's base

MATERIALS AND METHODS

Cell culture and treatment

The THP-1 cell line was obtained from Istituto Zooprofilattico (Brescia, Italy). The cell culture medium (RPMI-1640) was from Euroclone Diagnostica SpA (Pero, Milano, Italy) and the other substances were from Sigma-Aldrich Co. (St. Louis, MO, USA). First of all, THP-1 cells were diluted to 10^6 cells/mL in RPMI 1640 containing 2 mM L-glutamine, 50 µM 2-mercaptoethanol, 0.1 mg/mL streptomycin, 100 IU/mL penicillin, and 10% heated-inactivated fetal calf serum. The cells were cultured at 37°C in a 5% CO_2 incubator. The medium was changed every 2-3 days. The cells were treated with BB (Santa Cruz Biotechnology Inc., Dallas, TX, USA; CAS N° 20048-27-5) at a concentration of 1 µg/mL in dimethyl sulfoxide (DMSO) for 24, 48, and 72 h. The dose was selected from the results of cytotoxicity assays. Cells treated with only DMSO were used as a control (0.2% final concentration).

Total RNA extraction and complementary DNA (cDNA) synthesis

For total RNA extraction, after the treatment, the cells were centrifuged for 5 min at 1200 rpm at room temperature. Then the culture medium was discarded and cell pellets lysed with 700 µL of TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA). Next, a Quiagen miRNeasy mini kit was used to extract total RNA from the cells following the manufacturer's procedure. The purity and quantity of the total RNA were analyzed with a NanoDrop instrument (NanoVue Plus). After the RNA concentrations were determined, cDNA was synthesized by using a Qiagen script miRNA polymerase chain reaction (PCR) array kit according to the manufacturer's protocol. Then 0.25 µg and 2.0 µg of total RNA were retrotranscribed for miRNA expression profile and real-time (RT) PCR analysis, respectively.

miRNA expression profiling

After retrotranscription, miRNA expression profiling (including 86 miRNAs) was determined with Qiagen miScript miRNA PCR Arrays following the manufacturer's procedure with an ABI Prism® 7000 Sequence Detection System. The miRNA expression profiling was done in cells treated with BB (1 µg/mL) or DMSO as the control for 24 h. The amplification conditions consisted of an initial activation at 95°C for 15 min, then 40 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and the extension step for 30 s at 70°C. The data were analyzed with miScript miRNA PCR Arrays (http://pcrdataanalysis.sabiosciences.com/mirna)

Real-time PCR for detection of mature miRNA or noncoding RNA

To confirm the expression profile of miRNA, miR-155 and miR-21 were selected as immune system-associated miRNAs,

and their expression was evaluated by RT-PCR using an ABI Prism® 7000 Sequence Detection System with the same amplification conditions as miRNA expression profiling using the miScript SYBR Green PCR Kit (provided by Qiagen). Small nucleolar RNA61 (SNORD61), SNORD68, SNORD72, SNORD95, SNORD96A, and RNA, U6 small nuclear 6, pseudogene (RNU 6P) were used as endogenous miRNA controls in every reaction. All PCR reactions were performed in duplicate in a total of 25 µL of reaction volume. In the RT-PCR assay, the evaluation was done by calculation of the fluorescent signal. The cycle threshold (Ct) was the required number of cycles for the fluorescent signal to overshoot the threshold, which means to exceed the background level. Ct levels are conversely proportional to the quantity of target nucleic acid in the sample. When normalized gene expression in the test sample is divided by normalized gene expression in the control, fold-change (2-ΔΔCT) values are obtained, with values bigger than one indicating a positive or an upregulation and less than one indicating a negative or downregulation.[13]

Statistical analysis

The data were analyzed by SPSS version 23.0 (SPSS, Chicago, IL, USA). Normal distribution and homogeneity of the variances were evaluated by Shapiro-Wilk and Levene's tests, respectively. Student's t-test was used for the comparisons and p values less than 0.05 were considered statistically significant.

RESULTS

miRNA expression profiling

The expression profile of miRNA was evaluated in THP-1 cells treated for 24 h with BB (1 µg/mL) or DMSO as control. As shown in Figures 2 and 3, the expression of 32 miRNAs was upregulated and the expression of 31 miRNAs was downregulated in the cells after treatment with BB.

Real-time PCR for the detection of miRNAs

Following a search of the literature, we focused on miR-21 and miR-155 because these are thought to be involved in immune responses. THP-1 cells were exposed to BB (1 µg/mL) or DMSO as a control for 24, 48, and 72 h. After miRNA extraction, miR-21 and miR-155 levels were evaluated by RT-PCR. As shown in Figures 4 and 5, the expression of miR-21 was downregulated and the expression of miR-155 was upregulated, confirming the miRNA expression profile data.

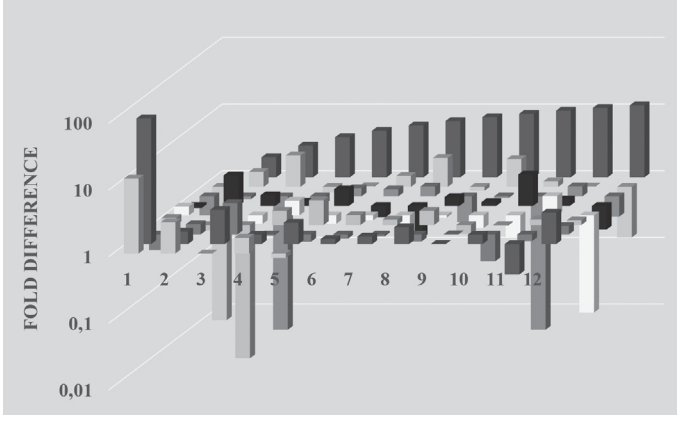

Figure 2. 3D profile of miRNA expression
miRNA: MicroRNA

DISCUSSION

ACD is a significant disease that occurs after topical exposure to low molecular weight chemicals.[14,15] It is a delayed-type hypersensitivity reaction that needs previous sensitization by the same chemical.[16] It is crucial to identify potential sensitizing agents because ACD is a common and serious health problem worldwide.[17]

It has been shown that miRNAs are involved in the processes of innate and adaptive immune systems. Among all the miRNAs, miR-21, miR-146a, and miR-155 are focused on by scientists. Signal transducer and activator of transcription-3 and nuclear factor kappa-B (NF-κB) regulate the expression of miR-21.[18] miR-155 is involved especially in proinflammatory processes. It has been observed that the expression of miR-21 is increased by

Upregulated miRNAs	Down-regulated miRNAs
hsa-miR-101-3p	hsa-miR-23a-3p
hsa-miR-223-3p	hsa-let-7g-5p
hsa-miR-30d-5p	hsa-miR-15a-5p
hsa-let-7f-5p	hsa-miR-374a-5p
hsa-miR-32-5p	hsa-miR-425-5p
hsa-miR-30a-5p	hsa-miR-320a
hsa-miR-28-5p	hsa-miR-124-3p
hsa-miR-155-5p	hsa-miR-21-5p
hsa-miR-25-3p	hsa-miR-30c-5p
hsa-miR-24-3p	hsa-miR-191-5p
hsa-miR-22-3p	hsa-let-7a-5p
hsa-miR-181a-5p	hsa-miR-210-3p
hsa-miR-125a-5p	hsa-miR-29a-3p
hsa-miR-140-3p	hsa-miR-19a-3p
hsa-miR-7-5p	hsa-miR-142-3p
hsa-miR-424-5p	hsa-let-7b-5p
hsa-miR-30b-5p	hsa-miR-93-5p
hsa-let-7i-5p	hsa-miR-27a-3p
hsa-miR-15b-5p	hsa-let-7e-5p
hsa-let-7d-5p	hsa-miR-27b-3p
hsa-let-7c-5p	hsa-miR-18a-5p
hsa-miR-126-3p	hsa-miR-16-5p
hsa-miR-23b-3p	hsa-miR-19b-3p
hsa-miR-141-3p	hsa-miR-423-5p
hsa-miR-9-5p	hsa-miR-30e-5p
hsa-miR-20a-5p	hsa-miR-106a-5p
hsa-miR-92a-3p	hsa-miR-17-5p
hsa-miR-128-3p	hsa-miR-181b-5p

Figure 3. Upregulated and downregulated miRNAs
miRNAs: MicroRNAs

vesicular stomatitis virus infection in macrophages.[19] Moreover, miR-155 can suppress the SH2 domain-containing inositol-5-phosphatase that can lead to activation of Akt kinase and upregulation of interferon response genes during the cellular response to lipopolysaccharide.[20] In an animal study, it was demonstrated that miR-155 stimulated atherosclerosis in mice via directly suppressing B-cell lymphoma 6 protein, which is a transcription factor that attenuates NF-κB signaling.[21] miR-155 is also involved in the development and activation of adaptive immune cells such as effector T-cell subsets.[22]

In the present study, we observed upregulation and downregulation in the expression of miR-155 and miR-21 in THP-1 cells after exposure to BB, respectively. The up- and downregulation of the miRNAs were validated by RT-PCR. Similarly, in a study by Sonkoly et al.[23] the expression of miR-155 was found to be highest in the skin samples from patients with atopic dermatitis compared to healthy controls. It was also observed that after the topical exposure of relevant allergens to nonlesional skin of atopic dermatitis patients, miR-155 expression was induced. Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), a negative regulator of T-cell function, has been repressed by miR-155 and in animal models blocking of CTLA-4 stimulated a much severe allergic reaction and inflammation by increasing the number of eosinophils and immunoglobulin-E, while increases in the expression of CTLA-4 ameliorated the symptoms of allergic pulmonary inflammation in humans.[24,25] Furthermore, in patients with atopic dermatitis miR-155 has significantly higher expression compared to in healthy subjects and the levels of expression correlated with the severity of atopic dermatitis.[26] In contrast to these studies, miR-155 levels were lower in sputum from allergic asthmatics than in healthy subjects.[27]

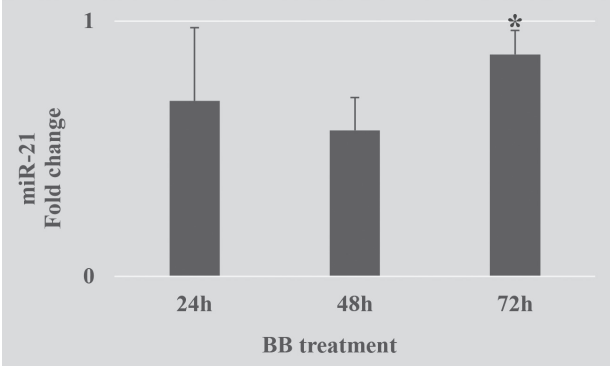

Figure 4. Expression of miR-21 in THP-1 cells following exposure to BB (1 µg/mL) or DMSO for 24, 48, and 72 h. Fold changes in miR-21 were calculated for each sample for each time point and expressed as mean ± standard deviation, *p<0.05 vs. control at the different time points of three independent experiments
BB: Bandrowski's base, DMSO: Dimethyl sulfoxide

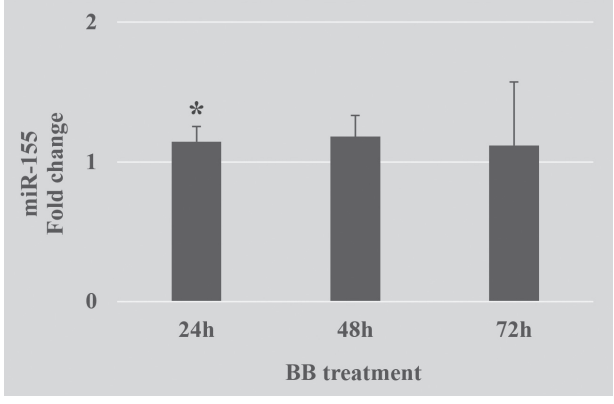

Figure 5. Expression of miR-155 in THP-1 cells following exposure to BB (1 µg/mL) or DMSO for 24, 48, and 72 h. Fold changes in miR-21 were calculated for each sample for each time point and expressed as mean ± standard deviation, *p<0.05 vs. control at the different time points of three independent experiments
BB: Bandrowski's base, DMSO: Dimethyl sulfoxide

miR-21 levels were evaluated in a number of studies. In line with the results obtained from the present study, it was shown that miR-21 was lower in monocytes from children with allergic rhinitis and in patients with metabolic syndrome.[28,29] In contrast, in an animal study, miR-21 levels were higher in the skin of mice with contact dermatitis and in esophageal tissue in mice with eosinophilic esophagitis, which can reflect interspecies differences between mice and humans in miRNA expression.[30,31]

In addition to their involvement in various biological processes, miRNAs have potential in disease diagnostics and therapies. Due to their stability, miRNAs could be used as biomarkers. Currently, miRNA panels are used by clinicians in order to determine the origins of cancer cells. The development of miRNA therapeutics has proved more challenging because of delivery problems. In addition, relations between gene expression and miRNAs are complex. As a result, administration or silencing of one miRNA could modify the expression of numerous genes with unknown consequences. In multifactorial diseases, successful silencing of a single gene may not be efficient in clinical practice. Therefore, there is only one miRNA drug in clinical trials (SPC3649: inhibitor/antagomir of miR-122, Santaris Pharma, Denmark) and several more are waiting to enter the clinical phase.[32] From this point of view, our study has some limitations. We evaluated only the gene expression profile but protein products made from genes and other immune systems biomarkers such as interleukins and interferons should also be analyzed. These are our aims for future projects.

CONCLUSION

Our results showed that miR-155 expression was upregulated and miR-21 expression was downregulated in THP-1 cells treated with BB. It has been demonstrated that miRNAs are involved in many biological processes and they are important in the pathogenesis of allergic inflammation. However, relations between gene expression and miRNAs are complex and so further investigation of these preliminary results is needed to explain the precise functional roles of these two miRNAs in the regulation of chemical allergen potency and also their potential to serve as novel therapeutic targets.

Conflict of interest: The authors declare that they have no conflicts of interest.

REFERENCES

1. Corsini E, Roggen EL. Immunotoxicology: opportunities for non-animal test development. Altern Lab Anim. 2009;37:387-397.
2. Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population--prevalence and main findings. Contact Dermatitis. 2007;57:287-299.
3. Galbiati V, Papale A, Marinovich M, Gibbs S, Roggen E, Corsini E. Development of an *in vitro* method to estimate the sensitization induction level of contact allergens. Toxicol Lett. 2017;271:1-11.
4. White JM, Kullavanijaya P, Duangdeeden I, Zazzeroni R, Gilmour NJ, Basketter DA, McFadden JP. p-Phenylenediamine allergy: the role of Bandrowski's base. Clin Exp Allergy. 2006;36:1289-1293.
5. Chanput W, Mes JJ, Wichers HJ. THP-1 cell line: an *in vitro* cell model for immune modulation approach. Int Immunopharmacol. 2014;23:37-45.
6. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer. 1980;26:171-176.
7. Galbiati V, Mitjans M, Corsini E. Present and future of *in vitro* immunotoxicology in drug development. J Immunotoxicol. 2010;7:255-267.
8. Wiemer EA. miRNAs and cancer. J RNAi Gene Silencing. 2006;2:173-174.
9. Sonkoly E, Pivarcsi A. microRNAs in inflammation. Int Rev Immunol. 2009;28:535-561.
10. Calame K. MicroRNA-155 function in B Cells. Immunity. 2007;27:825-827.
11. Sheedy FJ. Turning 21: Induction of miR-21 as a Key Switch in the Inflammatory Response. Front Immunol. 2015;6:19.
12. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281-297.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-408.
14. Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population--prevalence and main findings. Contact Dermatitis. 2007;57:287-299.
15. Peiser M, Tralau T, Heidler J, Api AM, Arts JHE, Basketter DA, English J, Diepgen TL, Fuhlbrigge RC, Gaspari AA, Johansen JD, Karlberg AT, Kimber I, Lepoittevin JP, Liebsch M, Maibach HI, Martin SF, Merk HF, Platzek T, Rustemeyer T, Schnuch A, Vandebriel RJ, R White I, Luch A. Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. Current knowledge assembled at an international workshop at BfR, Germany. Cell Mol Life Sci. 2012;69:763-781.
16. Basketter D, Darlenski R, Fluhr JW. Skin irritation and sensitization: mechanisms and new approaches for risk assessment. Skin Pharmacol Physiol. 2008;21:191-202.
17. Papale A, Kummer E, Galbiati V, Marinovich M, Galli CL, Corsini E. Understanding chemical allergen potency: role of NLRP12 and Blimp-1 in the induction of IL-18 in human keratinocytes. Arch Toxicol. 2017;91:1783-1794.
18. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell. 2010;39:493-506.
19. Wang P, Hou J, Lin L, Wang C, Liu X, Li D, Ma F, Wang Z, Cao X. Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1. J Immunol. 2010;185:6226-6233.
20. O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci U S A. 2009;106:7113-7118.
21. Nazari-Jahantigh M, Wei Y, Noels H, Akhtar S, Zhou Z, Koenen RR, Heyll K, Gremse F, Kiessling F, Grommes J, Weber C, Schober A. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. J Clin Invest. 2012;122:4190-4202.
22. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316:608-611.
23. Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, Xu N, Meisgen F, Wei T, Bradley M, Stenvang J, Kauppinen S, Alenius H, Lauerma A, Homey B, Winqvist O, Ståhle M, Pivarcsi A. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. J Allergy Clin Immunol. 2010;126:581-9.e20.
24. Hellings PW, Vandenberghe P, Kasran A, Coorevits L, Overbergh L, Mathieu C, Ceuppens JL. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. Eur J Immunol. 2002;32:585-594.
25. Abrams JR, Lebwohl MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, Menter A, Lowe NJ, Krueger G, Brown MJ, Weiner RS, Birkhofer MJ, Warner GL, Berry KK, Linsley PS, Krueger JG, Ochs HD, Kelley SL, Kang S. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. J Clin Invest. 1999;103:1243-1252.
26. Ma L, Xue HB, Wang F, Shu CM, Zhang JH. MicroRNA-155 may be involved in the pathogenesis of atopic dermatitis by modulating the differentiation and function of T helper type 17 (Th17) cells. Clin Exp Immunol. 2015;181:142-149.
27. Lu TX, Munitz A, Rothenberg ME. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. J Immunol. 2009;182:4994-5002.
28. Chen RF, Huang HC, Ou CY, Hsu TY, Chuang H, Chang JC, Wang L, Kuo H-C, Yang KD. MicroRNA-21 expression in neonatal blood associated with antenatal immunoglobulin E production and development of allergic rhinitis. Clin Exp Allergy. 2010;40:1482-1490.
29. He QF, Wang LX, Zhong JM, Hu RY, Fang L, Wang H, Gong WW, Zhang J, Pan J, Yu M. Circulating MicroRNA-21 is Downregulated in Patients with Metabolic Syndrome. Biomed Environ Sci. 2016;29:385-389.
30. Vennegaard MT, Bonefeld CM, Hagedorn PH, Bangsgaard N, Løvendorf MB, Odum N, Woetmann A, Geisler C, Skov L. Allergic contact dermatitis induces upregulation of identical microRNAs in humans and mice. Contact Dermatitis. 2012;67:298-305.
31. Lu TX, Sherrill JD, Wen T, Plassard AJ, Besse JA, Abonia JP, Franciosi JP, Putnam PE, Eby M, Martin LJ, Aronow BJ, Rothenberg ME. MicroRNA signature in patients with eosinophilic esophagitis, reversibility with glucocorticoids, and assessment as disease biomarkers. J Allergy Clin Immunol. 2012;129:1064-75.e9.
32. Hydbring P, Badalian-Very G. Clinical applications of microRNAs. F1000Res. 2013;2:136.