European Review for Medical and Pharmacological Sciences 2020; 24: 11804-11809

The potential role of microRNAs as biomarkers in atopic dermatitis: a systematic review

R.T. HERNÁNDEZ-RODRÍGUEZ[1], L.M. AMEZCUA-GUERRA[2,3]

[1]School of Medicine, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Immunology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico
[3]Health Care, Universidad Autónoma Metropolitana–Xochimilco, Mexico City, Mexico

Abstract. – OBJECTIVE: Reliable biomarkers are required for clinical use in atopic dermatitis (AD). MicroRNAs are mediators of post-transcriptional gene silencing, and specific expression patterns are being characterized in AD. To assess whether microRNAs could be useful biomarkers for clinical use in patients with AD.

MATERIALS AND METHODS: Systematic review of all articles identified in SCOPUS and PubMed through the PRISMA statement. Literature was summarized in narrative form and results are presented per category.

RESULTS: From a total of 118 identified references 11 manuscripts were included for qualitative analysis, after selecting them according to the eligibility criteria. An aberrant expression of microRNAs characterizes AD, which facilitates T cell polarization towards a Th17 phenotype, especially miR-155. There is also altered regulation of Th1/Th2 phenotypes by overexpression of miR-151a. The aberrant keratinocyte function observed in AD could also be due to altered expression of microRNAs, specifically miR-146a, miR-143 and miR-29.

Finally, miR-203 may reflect the extent of inflammation in AD, in parallel with the tumor necrosis factor pathway and immunoglobulin E levels.

CONCLUSIONS: MicroRNAs are easily identifiable molecules in a variety of cells and body fluids that may be useful as diagnostic (miR-155 and miR-146a) and disease severity (miR-203) biomarkers in patients with AD.

Key Words:
Atopic dermatitis, MicroRNA, Biomarkers.

Introduction

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder, commonly associated with asthma and allergic rhinitis[1]. Its prevalence is estimated to be 10% to 30% in children and 2% to 10% in adults, and predominantly affects individuals living in industrialized and urban areas[1,2]. Its pathophysiology remains elusive, although it appears to be the result of complex interactions between genetic factors, environmental triggers and immunological mechanisms[3]. Indeed, abnormalities in the production of filaggrin, ceramides and antimicrobial peptides cause skin barrier dysfunction, resulting in transepidermal water loss, dry skin and changes in the pH of the skin's surface. A deregulated skin barrier facilitates the penetration of allergens and pathogens, which eventually triggers inflammation. From an immunologic point of view, AD is characterized by a biphasic pattern, with T helper 2 (Th2)-type immune responses in acute skin lesions that include the production of cytokines, such as interleukin 4 (IL-4), IL-5 and IL-13, while a shift towards a Th1 response is found in chronic skin lesions, which is dominated by the production of interferon gamma (IFN-γ) and IL-12. Newer data suggest that all types of T cells may be expanded over time, and there may be a highly diverse repertoire with activation of Th17 and Th22 phenotypes, in addition to the aforementioned Th1 and Th2 responses. Finally, there is a characteristic production of chemoattractant molecules such as the chemokines C-C motif ligand 17 (CCL17) and 22 (CCL22), which play an important role in the eczematous reaction[4].

Recently, microRNAs (miRNA or miR) have emerged as key mediators of post-transcriptional gene silencing in inflammatory diseases, often called inflamma-miR. MiRNAs are unique non-coding RNA chains, of approximately 22 to 25 nucleotides, capable of negatively modulating gene expression by binding to the 3′ non-coding region (UTR) of multiple messenger RNAs (mRNA), which leads to degradation or translational repression of those mRNAs. Although miRNAs do not deactivate the expression of their

Corresponding Author: Luis M. Amezcua-Guerra, MD; e-mail: lmamezcuag@gmail.com

target genes, they reduce the number of mRNAs and proteins. The influence of a specific miRNA on the expression of a single mRNA may go unnoticed; however, because miRNAs are directed towards several mRNAs of the same pathway, a single miRNA can have a critical influence on a complete pathway. Since each miRNA may have hundreds of gene targets, they are able to modulate the production of a large amount of proteins. Among the functions regulated by different miRNAs are processes of differentiation, proliferation and activation of both keratinocytes and T cells[5].

Several miRNAs play a role in crosstalk between keratinocytes and immune cells, and a differentiated expression of miRNA profiles between patients with AD and healthy individuals has already been described. However, the measurement of miRNAs as biomarkers that may reflect the diagnosis and disease activity are not yet in clinical use. An adequate understanding of the role of miRNAs in AD may lead to the clarification of new pathogenic pathways and to develop useful knowledge in the fields of diagnosis and therapy. The objective of this study was to systematically review studies investigating the clinical role of miRNAs in patients with AD.

Materials and Methods

We searched all indexed articles in the SCOPUS and PubMed (MEDLINE) databases using the Medical Subject Heading (MeSH) "microRNAs" and "atopic dermatitis", while the keywords "atopic", "dermatitis", "microRNA" and "miRNA" were also used. In all searches we used "AND" as the Boolean operator. A single researcher (RTH-R) performed all electronic searches between December 1 and 8, 2018, with language restriction to English and Spanish, without a publication date limit and including studies from the inception and until November 30, 2018. Any original study that examined, in humans, the role of miRNAs in AD was included. We ruled out review articles and book chapters. We imported citations into the Excel program (Microsoft Office 97 for Windows) and manually deleted duplicated items. In the analysis of the manuscript title, all articles that satisfied the following were included: 1) studies that included patients with AD, and 2) studies with miRNA analysis in AD. In the abstract screening, those studies in which there was no analysis of any miRNA in AD were excluded. Finally, in the analysis of the full text, we excluded those manuscripts in which the role of at least one miRNA in humans with AD had not been studied. The systematic review was carried out under the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Since the present study did not include experimental subjects or identifiable private information, the protocol was not reviewed by the Local Ethics Committee.

Results

A total of 118 references were identified (96 from SCOPUS and 22 from PubMed). After removing 27 duplicates, 91 articles were eligible for title and abstract screening. Seventy-nine articles were eliminated during this phase and 12 articles were eligible for full-text reading. An additional article was excluded after full-text reading due to lack of data, and 11 articles were finally included in qualitative analysis. The flow chart is presented in Figure 1, while Table I summarizes the main studies and their findings.

Discussion

MiR-155

The miR-155 is an endogenous non-coding RNA of approximately 22 nucleotides, on chromosome 21q21.2[6]. A study on skin biopsies showed the existence of miR-155 overexpression during activation and differentiation of T cells in patients with AD. MiR-155 upregulation was associated with decreased levels of cytotoxic T-lymphocyte antigen 4 (CTLA-4), an important negative regulator of T cell activation that contributes to development of chronic skin inflammation[7]. The miR-155 is a proinflammatory factor in epithelial cells and a regulator of apoptosis by modulating CTLA-4, favoring the chronic induction of Th1 type responses in AD[8,9]. An interesting study in AD patients who did not received immunosuppressive drugs or desensitization therapy reported increased Th17 cells percentage, miR-155 expression, retinoic acid-related orphan receptor gamma t (RORγt) mRNA expression and IL-17 mRNA expression and plasma concentration in AD; conversely, mRNA expression and plasma concentration of the suppressor of cytokine signaling 1 (SOCS1) was decreased[10] . In addition, positive correlations were found between miR-155 expres-

sion and disease severity, Th17 cells percentage, RORγt mRNA expression and IL-17 mRNA expression and plasma concentrations.

Thus, miR-155 is overexpressed in AD and it may play an important role in the differentiation and functioning of Th17 cells by directly inhibiting SOCS1 activity. It is likely that miR-155 facilitates the activation and tissue infiltration by eosinophils in AD and other diseases such as asthma, reinforcing its participation in the pathophysiology of allergic diseases[5]. Besides its role as inflamma-miR, miR-155 can act as an onco-miR, favoring cell proliferation in processes such as cutaneous T cell lymphoma[11].

MiR-223

A study[12] sought for the expression of miR-223 in relation to prenatal exposure to tobacco smoke and the development of AD. The authors reported a relationship between miR-223 and elevated concentrations of urine cotinine (a marker used to measure exposure to tobacco). This miRNA was suggested to induce alterations in the fetal immune system, generating a higher susceptibility for the development of AD in children exposed *in utero* to tobacco smoke.

MiR-483-5p

The miR-483-5p modulates fibrogenesis through the regulation of collagen homeostasis. An analysis of serum and urine samples from AD patients found an association between high levels of serum miR-483-5p and elevated levels of immunoglobulin E and soluble type I (TNFRI) and type II (TNFRII) receptors for tumor necrosis factor[8].

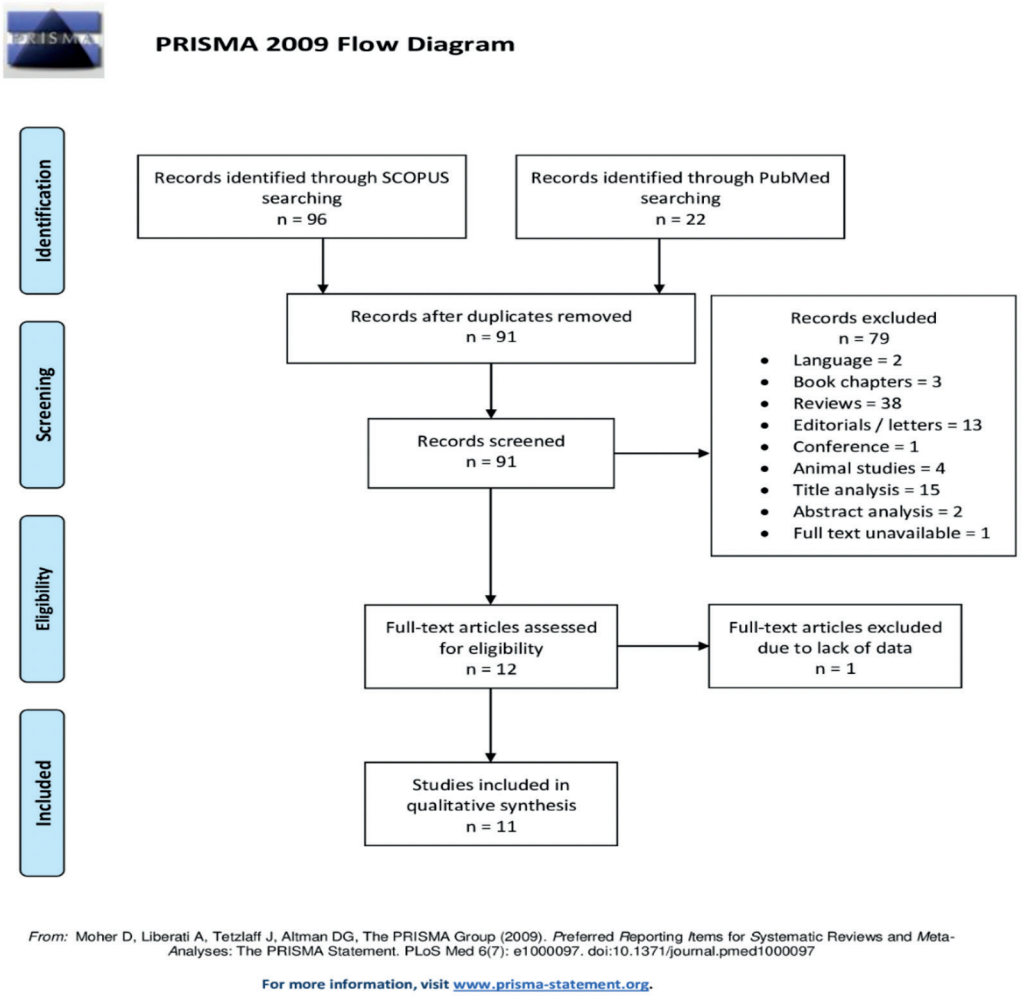

Figure 1. Flow chart of study selection according to the PRISMA statement.

Table I. Main studies on deregulated microRNA in atopic dermatitis (AD).

MicroRNA	Tissues or body fluid	Suggested target	Main finding	Reference
miR-151a	Plasma and serum	IL12RB2	Suppression of Th1 response with a shift towards Th2 response by inhibiting the expression of the *il12rb2* gene.	Chen et al[14]
miR-155	Plasma, skin	SOCS1	Elevated miR-155 expression was found in AD, and its levels correlated with disease severity, Th17 cells percentage, ROR-γt and IL-17 levels. The decrease in SOCS1 activity resulted in elevated levels of ROR-γt, which in turn resulted in an enhanced Th17 response.	Ma et al[10]
	Skin, peripheral blood cells	CTLA-4	miR-155 expression was upregulated in chronic skin lesions, predominantly in infiltrating immune cells. Overexpression of miR-155 in T cells resulted in decreased CTLA-4 levels and increased proliferative responses.	Sonkoly et al[7]
	Skin	CTLA-4	The level of miR-155 expression distinguishes AD patients from those with mycosis fungoides and cutaneous T-cell lymphoma, suggesting a relevant role for miR-155 as a regulator of cell proliferation in neoplastic processes (onco-miR).	Ralfkiaer et al[11]
miR-223	Maternal and cord blood	FOXP3	Maternal exposure to tobacco smoke during pregnancy correlates with the level of miR-223 expression in both maternal and cord blood. High expression of miR-223 was associated with a decrease in the number of regulatory T cells in maternal and cord blood. Children with low regulatory T cells at birth had an increased risk of AD during the first 3 years of life.	Herberth et al[12]
miR-483-5p	Serum and urine	MKNK1	MiR-483-5p was upregulated in serum of AD children, and its level was associated with other atopic conditions (rhinitis and asthma).	Lv et al[8]
miR-203	Serum and urine	SOCS-3, TNF, IL-24	MiR-203 was upregulated in serum from children with AD, in association with increased serum levels of tumor necrosis factor receptor I (TNFRI) and II (TNFRII). MiR-203 was downregulated in the urine of children with AD, and this was inversely associated with abnormal serum IgE levels.	Lv et al[8]
	Skin		MiR-203 expression was higher in skin samples from AD patients compared to samples from patients with mycosis fungoides.	Ralfkiaer et al[11]
miR-29b	Skin, serum	BCL2L2	MiR-29b was significantly upregulated in lesional skin and sera from AD patients, and miR-29b levels correlated with AD severity. Furthermore, miR-29b was critically involved in the apoptosis of keratinocytes by a mechanism dependent on the *bcl2l2* gene.	Gu et al[16]
miR-146a	Skin	NF-κB, IRAK1, CARD10	MiR-146a expression is increased in keratinocytes and chronic lesional skin from AD patients. In addition, miR-146a inhibited the expression of inflammatory molecules such as ubiquitin D, CCL5 and CCL8 chemokines in keratinocytes.	Rebane et al[18]
miR-143	Skin	IL-13Rα1	MiR-143 suppresses IL-13-dependent activity and inflammation through targeting IL-13Rα1 in epidermal keratinocytes, thereby modulating the production of epidermal barrier proteins such as filaggrin, loricrin, and involucrin.	Zeng et al[17]

MiR-203

Activation of keratinocytes results in the presence of miR-203, whose main target gene is the regulator of cytokine production SOCS-3[13]. Analysis in children with AD showed increased serum miR-203 levels, with a parallel response of the concentrations of TNFRI, TNFRII and immunoglobulin E. However, miR-203 was significantly decreased in the urine of AD children compared with healthy children, and urine miR-203 levels were significantly associated with abnormal serum immunoglobulin E levels. This suggests that urine miR-203 levels may be a useful biomarker to reflect the severity of inflammation in AD[8]. Recently, Ralfkiaer et al[11] compared the expression of miR-203 in skin biopsies from patients with AD, early fungoid mycosis and advanced cutaneous T-cell lymphoma. Notably, the authors observed that miR-203 was upregulated in AD and downregulated in fungoid mycosis and cutaneous lymphoma, suggesting a tumorigenesis suppressing effect for miR-203.

MiR-151a

An elegant study conducted in plasma and blood cell samples from 500 patients with AD and 200 healthy controls described that miR-151a is differentially expressed in the plasma of patients with AD. In transfected T cells, miR-151a was found to regulate the expression of the IL-12 receptor (IL12RB2) by targeting two loci in the 3' untranslated region of the *il12rb2* gene. Overexpression of miR-151a causes a decrease in the Th1 phenotype while favoring the Th2 response, a characteristic phenomenon of the chronic phase of AD[14].

MiR-29b

The miR-29b is predominantly located in the nucleus, acting as an element that directs nuclear functioning and is characteristically expressed at low levels, except in mitotic cells[15]. An experimental study[16] identified that miR-29b is one of the most significantly upregulated miRNAs in skin lesions from patients with AD compared to healthy controls, and its expression is associated with the development of AD. Furthermore, silencing of miR-29b was found to inhibit the IFN-γ-induced keratinocyte apoptosis, which could be attributed to the upregulation of its target gene, *Bcl2L2*. BCL2L2, as a member of the Bcl-2 family, is one of the key regulators of apoptosis. These results suggest that keratinocyte apoptosis, which is typically seen in AD, might be mediated at least partially through the miR-29b/BCL2L2 axis.

MiR-143

Interleukin 13 is a key Th2 cytokine that has been shown to impair the function of the epithelial barrier by inhibiting the production of several epidermal barrier proteins such as filaggrin, loricrin, and involucrin. In 2016, Zeng et al[17] demonstrated IL-13 as a potential target for miR-143, a miRNA known to be an important tumor suppressor. In this study, the expression of miR-143 was inhibited in human epidermal keratinocytes after stimulation with IL-13, while miR-143 was shown to negatively regulate the functioning of the IL-13 receptor (IL-13Rα1) by targeting its 3' UTR. In addition, IL-13 inhibited the expression of filaggrin, loricrin and involucrin in human keratinocytes, while forced expression of miR-143 eliminated the negative regulation induced by IL-13 on the production of epidermal barrier proteins. Together, these data suggest that miR-143 may suppress the inflammation induced by IL-13 in keratinocytes[5,17].

MiR-146a

In inflammatory skin conditions, CD4+ $CD25^{high}$ T cells, myeloid dendritic cells and mast cells express high levels of miR-146a. This abundant expression suggests that miR-146a could play an important role in the functioning of regulatory T cells during skin inflammation[13]. In AD, expression of miR-146a is increased in keratinocytes and chronic lesional skin, and it may inhibit the expression of chemoattractant molecules, such as CCL5 and CCL8. Moreover, *in vivo* and *in vitro* experiments show that miR-146a-mediated suppression in atopic skin inflammation occurs by blocking the signaling of nuclear factor κB (NF-κB), a pivotal mediator of inflammatory responses in innate and adaptive immunity[18].

Conclusions

AD is an inflammatory skin disease that displays a particular miRNA expression profile, which can lead to the development of novel disease markers and potential therapeutic targets. Acknowledging the absence of reliable markers for AD in clinical use, miRNAs are potential biomarkers since they are easily identifiable molecules in a variety of tissues, cells and body fluids. Most of the miRNAs studied in AD may be useful as diagnostic markers, with a special focus on miR-155 and miR-146a; meanwhile, miR-203 could be a marker of disease severity.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Ellis C, Luger T, Abeck D, Allen R, Graham-Brown RA, De Prost Y, Eichenfield LF, Ferrandiz C, Giannetti A, Hanifin J, Koo JY, Leung D, Lynde C, Ring J, Ruiz- Maldonado R, Saurat JH; ICCAD II Faculty. International Consensus Conference on Atopic Dermatitis II (ICCAD II): clinical update and current treatment strategies. Br J Dermatol 2003; 148: 3-10.
2) Huerta-Lopez JG, Del Olmo-Tellez H, Valdés-Brumer D. Prevalencia y factores asociados de rinitis alérgica y dermatitis atópica en niños. Alergia Asma Inmunol Pediatr 2008; 17: 54-64.
3) Carroll CL, Balkrishnan R, Feldman SR, Leischer AB, Manuel JC. The burden of atopic dermatitis: impact on the patient, family, and society. Pediatric Dermatology 2005; 22: 192-199.
4) Orciani M, Campanati A, Caffarini M, Ganzetti G, Consales V, Lucarini G, Offidani A, Di Primio R T helper (Th)1, Th17 and Th2 imbalance in mesenchymal stem cells of adult patients with atopic dermatitis: at the origin of the problem. Br J Dermatol 2017; 176: 1569-1576.
5) Dissanayake E, Inoue Y. MicroRNAs in Allergic Disease. Curr Allergy Asthma Rep 2016; 16: 1-8.
6) Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, Kroesen BJ, van den Berg A. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. J Pathol 2005; 207: 243-249.
7) 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.e1-20.
8) Lv Y, Qi R, Xu J, Di Z, Zheng H, Huo W, Zhang L, Chen H, Gao X. Profiling of serum and urinary microRNAs in children with atopic dermatitis. PLoS One 2014; 9: 1-16.
9) Rebane A, Akdis CA. MicroRNAs: Essential players in the regulation of inflammation. J Allergy Clin Immunol 2013; 132: 15-26.
10) 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.
11) Ralfkiaer U, Lindahl LM, Litman T, Gjerdrum LM, Ahler CB, Gniadecki R, Marstrand T, Fredholm S, Iversen L, Wasik MA, Bonefeld CM, Geisler C, Krejsgaard T, Glue C, Røpke MA, Woetmann A, Skov L, Grønbæk K, Odum N. MicroRNA expression in early mycosis fungoides is distinctly different from atopic dermatitis and advanced cutaneous T-Cell lymphoma. Anticancer Res 2014; 34: 7207-7217.
12) Herberth G, Bauer M, Gasch M, Hinz D, Röder S, Olek S, Kohajda T, Rolle-Kampczyk U, von Bergen M, Sack U, Borte M, Lehmann I; Lifestyle and Environmental Factors and Their Influence on Newborns Allergy Risk Study Group. Maternal and cord blood miR-223 expression associates with prenatal tobacco smoke exposure and low regulatory T-cell numbers. J Allergy Clin Immunol 2014; 133: 543-550.
13) Sonkoly E, Wei T, Janson PC, Sääf A, Lundeberg L, Tengvall-Linder M, Norstedt G, Alenius H, Homey B, Scheynius A, Ståhle M, Pivarcsi A. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One 2007; 2: e610.
14) Chen XF, Zhang LJ, Zhang J, Dou X, Shao Y, Jia XJ, Zhang W, Yu B. MiR-151a is involved in the pathogenesis of atopic dermatitis by regulating interleukin-12 receptor β2. Exp Dermatol 2018; 27: 427-432.
15) Hwang HW, Wentzel EA, Mendell JT. A hexanucleotide element directs microRNA nuclear import. Science 2007; 315: 97-100.
16) Gu Chaoying, Li Yin, Wu Jinfeng, Xu J. IFN-γ-induced microRNA-29b up-regulation contributes to keratinocyte apoptosis in atopic dermatitis through inhibiting Bcl2L2. Int J Clin Exp Pathol 2017; 10: 10117-10126.
17) Zeng YP, Nguyen GH, Jin HZ. MicroRNA-143 inhibits IL-13-induced dysregulation of the epidermal barrier-related proteins in skin keratinocytes via targeting to IL-13Rα1. Mol Cell Biochem 2016; 416: 63-70.
18) Rebane A, Runnel T, Aab A, Maslovskaja J, Rückert B, Zimmermann M, Plaas M, Kärner J, Treis A, Pihlap M, Haljasorg U, Hermann H, Nagy N, Kemeny L, Erm T, Kingo K, Li M, Boldin MP, Akdis CA. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. J Allergy Clin Immunol 2014; 134: 836-847.