Roles of MiR-31 and Endothelin-1 in Psoriasis vulgaris: Pathophysiological Functions and Potential Biomarkers

A running head: miR-31 and ET-1 in psoriasis patients

L. BORSKA,[1] C. ANDRYS,[2] M. CHMELAROVA,[3] H. KOVARIKOVA,[3] J. KREJSEK,[2] K. HAMAKOVA,[4] M. BERANEK,[2] V. PALICKA,[2] J. KREMLACEK,[1] P. BORSKY,[1] AND Z. FIALA[5]

[1] Institute of Pathological Physiology, Charles University, Faculty of Medicine, Simkova 870, 500 03 Hradec Kralove, Czech Republic
[2] Institute of Clinical Immunology and Allergology, Charles University, Faculty of Medicine Simkova 870, 500 03 Hradec Kralove, Czech Republic
[3] Institute of Clinical Biochemistry and Diagnostics, Charles University Hospital and Faculty of Medicine, Simkova 870, 500 03 Hradec Kralove, Czech Republic
[4] Department of Dermatology and Venereal Diseases, Charles University Hospital, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[5] Institute of Hygiene and Preventive Medicine, Charles University, Faculty of Medicine, Simkova 870, 500 03 Hradec Kralove, Czech Republic

***Corresponding author:**
Lenka Borska, Assoc. Prof., MD, PhD
Deputy Head
Institute of Pathological Physiology, Charles University, Faculty of Medicine
Simkova 870, 50038 Hradec Kralove, Czech Republic
E-mail: borka@lfhk.cuni.cz; Phone: +420-49-5816478, Fax: +420-49-5513597

Conflicts of interest: None are declared.

Approval and informed consent

The Ethics Committee of the University Hospital, Hradec Kralove, Czech Republic, approved the study. Informed written consent was obtained from each patient.

Summary

Psoriatic lesions are characterized by hyperproliferation, aberrant differentiation of keratinocytes resistant to apoptosis and inflammation. miR-31 plays pro-proliferative, pro-differentiative and pro-inflammatory roles and modulates apoptosis in psoriatic keratinocytes. Endothelin 1 (ET-1) is produced by psoriatic keratinocytes and suppresses apoptosis. Inflammation increases the production of ET-1, which in turn leads to the chronic stimulation of keratinocyte proliferation. The aim of this study was to identify the putative link between two potential biomarkers (miR-31 and ET-1) in patients with psoriasis. The study design included experimental group (29 patients with psoriasis), and the control group (22 blood donors). The PASI score evaluated the state of the disease (median: 18.6; interquartile range 14.5-20.9).

- Both, the serum level of ET-1 and the whole blood level of miR-31 were significantly increased ($p<0.001$ and $p<0.05$, respectively) in patients compared to the controls. However, a significant negative relationship between ET-1 and miR-31 was observed (Spearman's rho = -037, $p = 0.05$). It is possible that a negative feedback loop will be present between miR-31 and ET-1. Our results indicate that miR-31 and ET-1, potential biomarkers of the disease, play significant roles in the pathophysiology of psoriasis.

Keywords

miR-31; Endothelin-1; Keratinocyte; Psoriasis

Introduction

Psoriasis vulgaris is a multifactorial autoimmune disease with a prevalence of 2-3% in Europeans (Parisi *et al.* 2013). The pathogenesis of psoriasis is complex and the exact mechanism remains elusive. The disease is thought to result from a combination of genetic, epigenetic, and environmental variables. Psoriasis affects primarily the skin and/or joints and

is characterized by immunopathological inflammation, angiogenesis and keratinocyte hyperproliferation (Deng *et al.* 2016, Kondelkova *et al.* 2015). Keratinocytes derived from psoriatic plaques are resistant to induction of apoptosis as compared to keratinocytes derived from normal skin (Lerman *et al.* 2011).

Endothelins (ETs) comprise a family of four endogenous peptides (ET-1, ET-2, ET-3 and ET-4) (Olender *et al.* 2016). Numerous studies have described their pleiotropic biological activities, which contribute to the pathophysiology of numerous diseases (Lewicki et al. 2015, Nishiyama et al. 2016, Olender *et al.* 2016). ET-1 is a 21 amino acid peptide that is described as a vasoconstrictor, autocrine/paracrine mitogen and suppressor of apoptosis in the human body (de Miguel *et al.* 2015, Shichiri *et al*. 2000, Tsai *et al.* 2015). Endothelin-1 (ET-1) is produced in psoriatic keratinocytes and acts as an autocrine growth factor for these cells (Simeone *et al.* 2004).

MicroRNAs (miRNAs) are small noncoding RNAs of 19-24 nucleotides in length that function as posttranscriptional modulators of mRNA expression by suppressing mRNA translation or enhancing mRNA degradation (Amr *et al.* 2016). Abnormal regulation of miRNA expression has been implicated in the pathogenesis of several diseases, including chronic inflammatory disorders such as psoriasis (Wang *et al*. 2016, Wang *et al*. 2017, Stepicheva and Song 2016). MicroRNAs have also been suggested as potential biomarkers of immunopathological inflammation (Wu and Chen 2016).

miR-31 is a highly conserved miRNA that has been involved in both normal physiological processes (e.g., immune system functions) as well as in pathological processes (e.g., skin homeostasis) in various diseases, including oncologic and inflammatory diseases (e.g., psoriasis) (Liu *et al.* 2015, Schneider 2012, Stepicheva and Song 2016, Wang *et al.* 2016). This high degree of complexity may be caused by many molecular targets of miR-31 (Peng *et al.* 2012, Xu *et al.* 2013, Yan *et al.* 2015, Yan *et al.* 2015). miR-31 regulates diverse cellular and developmental processes by targeting genes involved in cell proliferation, apoptosis, cell differentiation, and cell motility (Stepicheva and Song 2016, Schneider 2012).

The aim of the current study was to identify the putative link between two potential biomarkers (miR-31 and endothelin-1) in psoriasis. The results provide new insights into the molecular basis of immunopathological inflammatory reactions in patients with psoriasis.

Methods

Observed groups and collection of biological samples

Our work included two groups of people. The experimental group consisted of 29 patients with psoriasis, and the control group consisted of 22 healthy blood donors.

Patients with active plaque psoriasis were selected to be included in the study between 2014 and 2016 at the Department of Dermatology and Venereology, University Hospital, Hradec Kralove. The group consisted of 15 women and 14 men (average age: 46 years; age range: 18–69 years; 18 smokers and 11 nonsmokers). Subjects with infections or other inflammatory diseases were excluded from the study. Similarly, patients suffering from psoriatic arthritis were excluded. The patients were not treated with any drugs that affect inflammatory reactions. The control group (CG) consisted of 11 women and 11 men (average age: 46 years; age range: 45-59 years; 6 smokers and 16 non-smokers).

Two samples of peripheral blood (with and without the anticoagulant) were collected from the cubital vein of all persons in both groups (BD Vacutainer sampling tubes; VACUETTE®, K3, EDTA; VACUETTE® LH 68). Blood serum was subsequently isolated by centrifugation (from one sample). Whole blood and blood serum samples were stored under minus 70°C until analysis. Repeated thawing and freezing cycles were avoided.

The Ethics Committee of the University Hospital in Hradec Kralove, Czech Republic approved our work. Informed written consent was obtained from each person in both of the studied groups.

Psoriasis Area and Severity Index (PASI score)

The state of disease was evaluated according the basic characteristics of actual disease status (erythema, desquamation, and skin infiltration) and expressed as the Psoriasis Area and Severity Index (PASI) score (Malkic Salihbegovic *et al.* 2015).

Serum levels of Endothelin-1 (ET-1)

Before analysis, the serum samples were diluted by a ratio of 1:2. The levels of ET-1 were detected by a Luminex bead-based multiplex assay, using magnetic beads and a premixed multi-analysis kit (R&D Systems USA). The detection range was 4.1 – 7.760 pg/ml. The fluorescence value was read by a Bio-Plex 200 analyzer (Bio-Rad, USA).

Whole blood miR-31 expression

The total amount of RNA, including miRNAs, was purified from 100 µl of the whole blood sample using Direct-zol™ RNA MiniPrep (Zymo Research, Irvine, CA, USA). RNA was subsequently eluted in 30 µl of DNase/RNase-Free water and RNA concentration and

purity were determined spectrophotometrically using a NanoDrop ND 1000 (Thermo Fisher Scientific, Wilmington, DE, USA). Optical densities were measured at 260 nm and 280 nm, and a A260/280 ratio was calculated. After the isolation, the samples were immediately processed or stored at -70°C.

Synthesis of cDNA was performed using TaqMan® Advanced miRNA cDNA Synthesis Kit with universal reverse transcription primers (Applied Biosystems, Foster City, CA, USA). We followed the manufacturer's protocol and used 8-10 ng of the total RNA for the reaction. Real-time PCR was performed using a Rotor Gene Q (Qiagen, Hilden, Germany), TaqMan® Fast Advanced Master Mix (Applied Biosystems) and specific TaqMan® Advanced miRNA Assays (Applied Biosystems). Assay hsa-miR-31-5p was used for miR-31 detection, and assay hsa-miR-361-5p was used for miR-361 detection; the latter served as an endogenous control.

All real-time PCR reactions were performed in triplicate, and the reaction volume was 10 µl, including 2.5 µl of the sample. The reaction conditions were set according to the manufacturer's protocol. The protocol involved enzyme activation at 95°C for 20 seconds followed by 40 cycles of both denaturation at 95°C for 3 seconds and annealing/extension at 60°C for 30 seconds. Obtained data were analyzed using the Rotor-Gene Q Series Software. Relative expression of each miRNA was determined using the $2^{-\Delta\Delta Ct}$ method with expression levels of miR-361 used for data normalization.

Statistical Analysis

Statistical analyses were performed using R software version 3.3.2 with the "nortest" and "psych" packages (R Core Team 2016). Because the Anderson-Darling test for the normality of the data had rejected the hypothesis of a normal distribution, nonparametric tests were used. The association between selected parameters was evaluated by Spearman Rank Order Correlations, and intergroup differences were evaluated using the Wilcoxon test. The results were considered to be statistically significant when the probability level (p) of a Type I error was below the alpha level of 0.05.

Results

The median of PASI score for the group of patients was 18.6 (interquartile range: 14.5 – 20.9). The statistical analysis of the results showed that ET-1 serum levels in patients were significantly higher (median: 6.44; interquartile range 6.24 – 6.93) than levels measured in the

healthy controls (median: 5.80; interquartile range 5.41 – 6.05; p<0.001). Similarly, whole blood miR-31 in the group of patients was significantly overexpressed (median: 0.52; interquartile range: 0.28 – 1.28; p<0.05), when compared to that measured in the group of healthy controls (median: -0.17; interquartile range: -0.66 – 0.64),

We found a significant negative relationship between ET-1 and miR-31 in the group of patients (Spearman's rho = "-0.37", p = 0.050). The relationship between ET-1 and miR-31 in the group of controls was not significant (Spearman's rho = -0.13, p = 0.556) (Fig. 1). In the group of patients, we did not find significant relationships between miR-31 and the PASI score (Spearman's rho = -0.240) or between ET-1 and the PASI score (Spearman's rho = 0.137).

Discussion

Psoriatic lesions are characterized by inflammation and hyperproliferation with aberrant differentiation of keratinocytes. The important role of keratinocytes in mediating the disease process is evidenced (Helwa *et al.* 2015).

Apoptosis, a genetically controlled cell death program (Shichiri *et al.* 2000), plays a pivotal role in the development, differentiation, and defense of skin homeostasis (Elango *et al.* 2016, Lerman *et al.* 2011). Recent data showed that hyperproliferation of keratinocytes and decreased epidermal keratinocyte apoptosis (via the action of inflammatory mediators) are principal clinical aspects of psoriasis (Deng *et al.* 2016, Elango *et al.* 2016, Lerman *et al.* 2011).

Keratinocytes of healthy persons produce cytokines/chemokines, variety of growth factors, and regulate skin barrier function (Smithrithee *et al.* 2015). Abnormalities in any of these processes may result in chronic disease, such as psoriasis. Keratinocytes from psoriatic epidermis express much higher levels of cytokines and growth factors (Lerman *et al.* 2011). Therefore, the drugs (biological therapy) that act by inhibiting these higher levels of cytokines have an important role in the treatment of these diseases (Deng *et al.* 2016). Some of the keratinocytes can stimulate cell proliferation through an autocrine loop stimulated by peptides such as ET-1 (Simeone *et al.* 2004). Control of this upregulated growth is an attractive mechanism for therapeutics (Simeone *et al.* 2004). Modulation of ET-1 may be the target of such therapy of psoriasis (Simeone *et al.* 2004).

In presented study we found significantly increased serum level of ET-1 in the psoriatic patients compared to the control group (p<0.001). Other authors present similar

results. The higher expression of ET-1 in biopsy samples of skin lesions was documented in inflammatory and neoplastic skin diseases with keratinocyte proliferation, including psoriasis (Salem *et al.* 2015). Other studies with psoriasis patients showed elevated levels of plasma endothelins ET-1 and ET-2 (Trevisan *et al.* 1994, Zachariae *et al.* 1996) and serum endothelin ET-1 (Bonifati et al. 1998).

We found significantly elevated whole blood level of miR-31 in the psoriatic patients compared to the control group ($p<0.05$). A number of studies have shown the important role of miRNAs in skin biology and diseases associated with the activation of the endothelin system. These diseases include cancer, kidney disease, cardiovascular diseases, inflammatory diseases, infectious diseases, and blood diseases, all of which may be aggravated by aberrant miRNAs expression (von Brandenstein *et al.* 2012, Feng *et al.* 2017).

According to Wang et al., the miRNAs play a regulatory role in inhibition of protein expression and altered expression of miRNAs can be associated with cancer development and progression. As these authors reported, downregulated expression of miR-31 is significantly associated with poor tumor differentiation, lymph node metastasis, advanced T stage and worse overall survival, suggesting that miR-31 have a suppressive role in progression of gastric cancer (Wang 2016). Several studies suggest that miR-31 activity can be disrupted during the onset and progression of inflammatory disorders like psoriasis (Li *et al.* 2015, Stepicheva and Song 2016).

Generally, it seems that ET-1 can affect apoptosis in the sense of its suppression, while miR-31 rather in the sense of its promotion. In our study we found negative correlation between elevated serum levels of ET-1 and upregulation of miR-31 (Spearman's rho = -037, $p = 0.05$) and we assume that a negative feedback loop may be present between miR-31 and ET-1. Van Gele *et al.* (2016) stated that the miRNAs could serve as biomarkers that predict disease predisposition or reflect the degree of illness (Van Gele *et al.* 2016). This information supports the assumption that the level of miRNAs is changing according to the condition of the disease. Our patients were examined in various stages of intensity of the acute phase of their disease (PASI score, interquartile range: 14.5 – 20.9), which could affect the situation in their balance among the factors influencing suppression and promotion of apoptosis (including the balance between influence of the ET-1 and the miR-31).

Despite all the existing data, we are still far from understanding the biological pathways targeted by miR-31 (how the miR-31 contributes to the regulation of the inflammatory response).

Conclusion

- Our results indicate that miR-31 and ET-1, potential biomarkers of the disease, play significant roles in the pathophysiology of psoriasis vulgaris.

Acknowledgments

The study was supported by Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic, project PROGRES Q40-09.. The authors acknowledge and thank Mgr. Dana Knajflova and American Journal Experts (Certificate Verification Key: 877B-0072-2435-C103-2DD2) for proofreading the text and helping with linguistics.

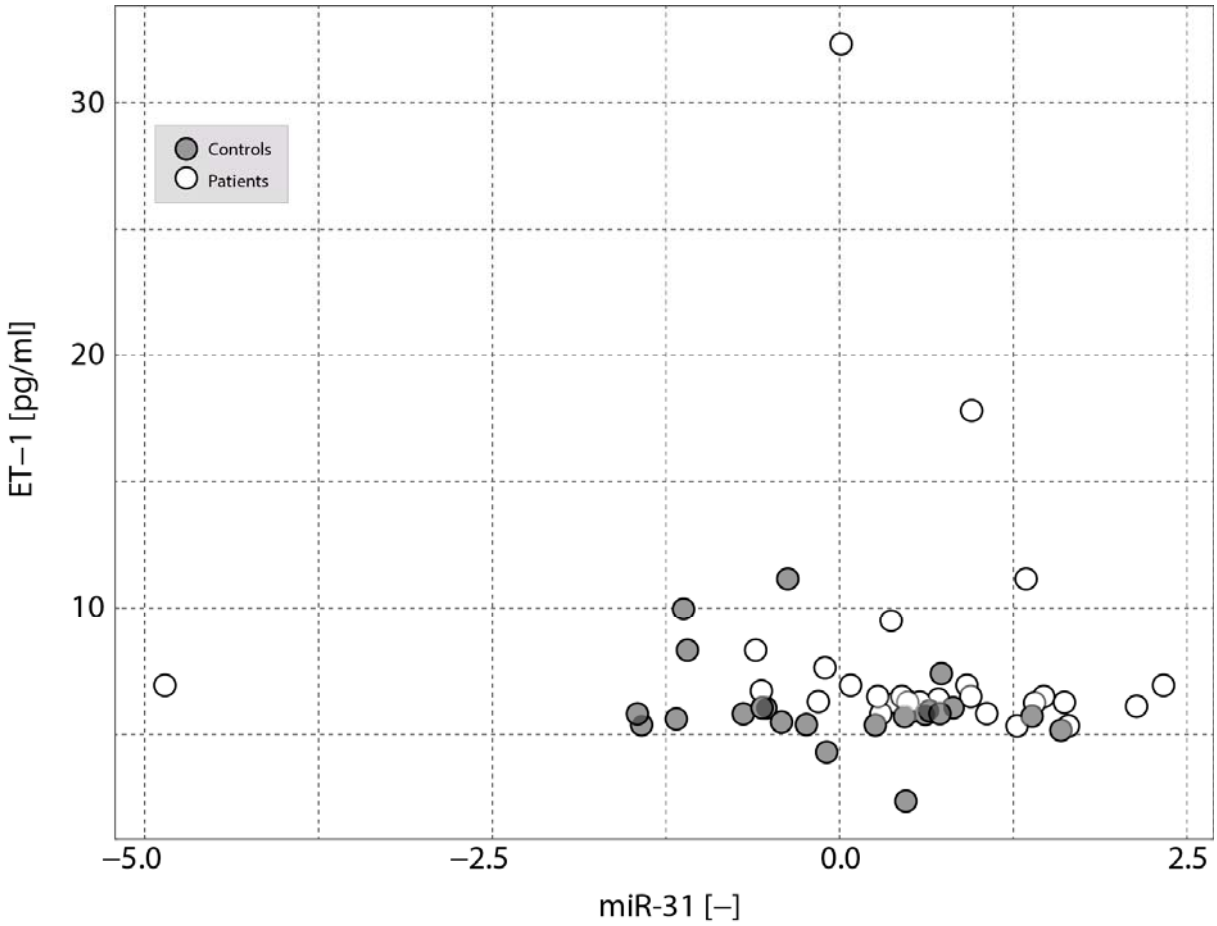

Figure 1. The scatter plot depicts the serum levels of endothelin-1 (ET-1) and miR-31 in patients with psoriasis (*n* = 29) and control subjects (*n* = 22). Each point represents a single subject (open circles: patients, dark circles: controls). While there was no significant relationship between ET-1 and miR-31 in controls (Spearman's rho = -0.13, p = 0.556), for patients, we found a significant negative correlation between ET-1 and miR-31 (Spearman's rho = -037, p = 0.05).

References

AMR KS, BAYOUMI FS, ELGENGEHY FT, ABDALLAH SO, AHMED HH, EISSA E: The role of microRNA-31 and microRNA-21 as regulatory biomarkers in the activation of T lymphocytes of Egyptian lupus patients. *Rheumatol Int* **36**: 1617-1625, 2016.

BONIFATI C, MUSSI A, CARDUCCI M, PITTARELLO A, D'AURIA L, VENUTI A, BAGNATO A, SALANI D, FAZIO M, AMEGLIO F: Endothelin-1 levels are increased in sera and lesional skin extracts of psoriatic patients and correlate with disease severity. *Acta Derm Venereol* **78:** 22-26, 1998

DE MIGUEL C, POLLOCK DM, POLLOCK JS: Endothelium-derived ET-1 and the development of renal injury. *Am J Physiol Regul Integr Comp Physio* **309**: R1071-1073, 2015.

DENG Y, CHANG C, LU Q: The Inflammatory Response in Psoriasis: a Comprehensive Review. *Clin Rev Allergy Immuno* **50**: 377-389, 2016.

ELANGO T, THIRUPATHI A, SUBRAMANIAN S, ETHIRAJ P, DAYALAN H, GNANARAJ P: Methotrexate treatment provokes apoptosis of proliferating keratinocyte in psoriasis patients. *Clin Exp Med* 2016 [Epub ahead of print]

FENG C, BAI M, YU NZ, WANG XJ, LIU Z: MicroRNA-181b negatively regulates the proliferation of human epidermal keratinocytes in psoriasis through targeting TLR4. *J Cell Mol Med* **21**: 278-285, 2017.

HELWA I, PATEL R, KAREMPELIS P, KADDOUR-DJEBBAR I, CHOUDHARY V, BOLLAG WB: The antipsoriatic agent monomethylfumarate has antiproliferative, prodifferentiative, and anti-inflammatory effects on keratinocytes. *J Pharmacol Exp Ther* **352**: 90-97, 2015.

KONDELKOVA K, BORSKA L, ANDRYS C, KREJSEK J, HAMAKOVA K, RENDAROVA S, REHACEK V, KREMLACEK J, FIALA Z: Selected Inflammatory and Metabolic Markers in Psoriatic Patients Treated with Goeckerman Therapy. *Mediators Inflamm* Article ID **979526**: 1-8, 2015.

LERMAN G, VOLMAN E, SIDI Y, AVNI D: Small-interfering RNA targeted at antiapoptotic mRNA increases keratinocyte sensitivity to apoptosis. *Br J Dermatol* **164**: 947-956, 2011.

LEWICKI L, SIEBERT J, MAREK-TRZONKOWSKA N, MASIEWICZ E, KOLINSKI T, REIWER-GOSTOMSKA M, TARGONSKI R, TRZONKOWSKI P: Elevated Serum

Tryptase and Endothelin in Patients with ST Segment Elevation Myocardial Infarction: Preliminary Report. *Mediators Inflamm* Article ID **395173**: 1-16, 2015.

LI D, LI X, WANG A, MEISGEN F, PIVARCSI A, SONKOLY E, STÅHLE M, LANDÉN NX: MicroRNA-31 Promotes Skin Wound Healing by Enhancing Keratinocyte Proliferation and Migration. *J Invest Dermatol* **135**: 1676-1685, 2015.

LIU D, SUN X, YE P: miR-31 Overexpression Exacerbates Atherosclerosis by Targeting NOX4 in apoE(-/-) Mice. *Clin Lab* **61**: 1617-1624, 2015.

MALKIC SALIHBEGOVIC E, HADZIGRAHIC N, CICKUSIC AJ: Psoriasis and metabolic syndrome. *Med Arch* **69**: 85-87, 2015.

NISHIYAMA SK, ZHAO J, WRAY DW, RICHARDSON RS: Vascular function and Endothelin-1: Tipping the Balance between Vasodilation and Vasoconstriction. *J Appl Physiol* (1985). **122**: 354-360, 2017.

OLENDER J, NOWAKOWSKA-ZAJDEL E, WALKIEWICZ K, MUC-WIERZGOŃ M: Endothelins and carcinogenesis. *Postepy Hig Med Dosw (Online)* 70: 872-880, 2016.

PARISI R, SYMMONS DP, GRIFFITHS CE, ASHCROFT DM; Identification and Management of Psoriasis and Associated ComorbidiTy (IMPACT) project team. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J Invest Dermatol* **133**: 377-385, 2013.

PENG H, KAPLAN N, HAMANAKA RB, KATSNELSON J, BLATT H, YANG W, HAO L, BRYAR PJ, JOHNSON RS, GETSIOS S, CHANDEL NS, LAVKER RM. MicroRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. *Proc Natl Acad Sci U S A* **109**: 14030-14034, 2012.

SALEM SA, GAMAL ALY D, SALAH YOUSSEF N, MONEIM EL-SHAER MA: Immunohistochemical assessment of endothelin-1 axis in psoriasis, basal cell carcinoma and squamous cell carcinoma. *G Ital Dermatol Venereol* **150**: 283-291, 2015.

SHICHIRI M, YOKOKURA M, MARUMO F, HIRATA Y: Endothelin-1 inhibits apoptosis of vascular smooth muscle cells induced by nitric oxide and serum deprivation via MAP kinase pathway. *Arterioscler Thromb Vasc Biol* **20**: 989-997, 2000.

SCHNEIDER MR: MicroRNAs as novel players in skin development, homeostasis and disease. *Br J Dermatol* **166**: 22-28, 2012.

SMITHRITHEE R, NIYONSABA F, KIATSURAYANON C, USHIO H, IKEDA S, OKUMURA K, OGAWA H: Human β-defensin-3 increases the expression of interleukin-37 through CCR6 in human keratinocytes. *J Dermatol Sci* **77**: 46-53, 2015.

STEPICHEVA NA, SONG JL. Function and regulation of microRNA-31 in development and disease. *Mol Reprod Dev* **83**: 654-674, 2016.

SIMEONE P, TESON M, LATINI A, CARDUCCI M, VENUTI A: Endothelin-1 could be one of the targets of psoriasis therapy. *Br J Dermatol* **151**: 1273-1275, 2004.

TREVISAN G, STINCO G, GIANSANTE C, FIOTTI N, VIDIMARI P, KOKELJ F: Psoriasis and endothelins. *Acta Derm Venereol Suppl (Stockh)* **186**: 139-140, 1994.

TSAI KW, HU LY, CHEN TW, LI SC, HO MR, YU SY, TU YT, CHEN WS, LAM HC: Emerging role of microRNAs in modulating endothelin-1 expression in gastric cancer. *Oncol Rep* **33**: 485-493, 2015.

VAN GELE M, BRACKE S, ALVES DE MEDEIROS AK, LAMBERT J: Exploring the feasibility of whole blood to identify systemic miRNA biomarkers for patients with moderate to severe psoriasis. *Eur J Dermatol* **26**: 195-198, 2016.

VON BRANDENSTEIN M, RICHTER C, FRIES JW: MicroRNAs: Small but amazing, and their association with endothelin. *Life Sci* **91**: 475-489, 2012.

WANG H, ZHANG X, LIU Y, NI Z, LIN Y, DUAN Z, SHI Y, WANG G, LI F: Downregulated miR-31 level associates with poor prognosis of gastric cancer and its restoration suppresses tumor cell malignant phenotypes by inhibiting E2F2. *Oncotarget* **7**: 36577-36589, 2016.

WANG MJ, XU YY, HUANG RY, CHEN XM, CHEN HM, HAN L, YAN YH, LU CJ: Role of an imbalanced miRNAs axis in pathogenesis of psoriasis: novel perspectives based on review of the literature. *Oncotarget* **8**: 5498-5508, 2017.

WU T, CHEN G: MiRNAs Participate in MS Pathological Processes and Its Therapeutic Response. *Mediators Inflamm* Article ID **4578230**: 1-9, 2016.

XU N, MEISGEN F, BUTLER LM, HAN G, WANG XJ, SÖDERBERG-NAUCLÉR C, STÅHLE M, PIVARCSI A, SONKOLY E: MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40. *J Immunol* **190**: 678-688, 2013.

YAN S, XU Z, LOU F, ZHANG L, KE F, BAI J, LIU Z, LIU J, WANG H, ZHU H, SUN Y, CAI W, GAO Y, SU B, LI Q, YANG X, YU J, LAI Y, YU XZ, ZHENG Y, SHEN N, CHIN YE, WANG H: NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. *Nat Commun* **6**: 1-39, 2015.

YAN HJ, MA JY, WANG L, GU W: Expression and significance of circulating microRNA-31 in lung cancer patients. *Med Sci Monit* **9**: 722-726, 2015.

ZACHARIAE H, HEICKENDORFF L, BJERRING P: Plasma endothelin in psoriasis: possible relations to therapy and toxicity. *Acta Derm Venereol* **76**: 442-43, 1996.