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CONFLICT OF INTEREST
The authors declare no conflicts of interest related to the contents of this work.

Matija Rijavec[1,2]
Mitja Košnik[1,3]
Ana Koren[1]
Peter Kopač[1,3]
Julij Šelb[1]
Romana Vantur[1]
Žan Kogovšek[1]
Mojca Bizjak[1]
Nissera Bajrović[1]
Mihaela Zidarn[1,3]
Peter Korošec[1]

[1]*University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia*
[2]*Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3]*Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Correspondence
Matija Rijavec, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik 36, 4204 Golnik, Slovenia.
Email: matija.rijavec@klinika-golnik.si

ORCID
Matija Rijavec https://orcid.org/0000-0002-2596-4952
Mitja Košnik https://orcid.org/0000-0002-4701-7374
Ana Koren https://orcid.org/0000-0002-9671-1645
Mojca Bizjak https://orcid.org/0000-0003-2595-468X
Peter Korošec https://orcid.org/0000-0002-0835-1599

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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Identification of novel miRNA-mRNA regulatory networks in contact dermatitis by integrated microarray analysis

To the Editor,
Contact dermatitis (CD) is a major occupational disorder, characterized by pruritus and inflammation following direct topical exposure to an allergen or irritant.[1] The role of micro RNAs (miRNAs) in skin diseases, including CD, has been investigated due to their compelling potential applicability.[1] miRNAs are short single-stranded RNA molecules, which take part in the post-transcriptional regulation.[1] Most previously conducted studies have focused on changes in miRNA expression profiles[1-3]; however, their regulatory effects as well as changes in the miRNAome in response to contact sensitizers and irritants with different physiochemical properties needs elucidation.

Herein, we conducted an integrative analysis of miRNA/mRNA expression profiles in skin using same sample miRNA and mRNA expression data from positive patch test reactions from patients exposed to allergens (nickel sulfate (NI), epoxy resin (EP) and methylochloroisothiazolinone (MCI); each n = 5), irritants (sodium lauryl sulfate [SLS, n = 9] and nonanoic acid [NO, n = 5]) and from non-affected skin (baseline, n = 5). Allergens, compared to irritants, induced distinct miRNA signatures in human skin (Figure 1A,B), which is mirrored in the differentially expressed miRNAs in the different exposures (Figure 1C). Overall, allergen encounter induced similar "core" miRNAs. Contrary, for the irritants, only SLS induced differentially expressed miRNAs, majorly overlapping with allergen-induced differentially expressed miRNAs (Figure 1D). All differentially expressed miRNAs are listed in Figure S1 and highly relevant miRNAs are highlighted in Figure 1E.

Since allergens and irritants exert different molecular effects on human skin, we analyzed both entities separately. To integrate the miRNA and mRNA datasets, we employed canonical correlation analysis as a multivariate exploratory approach. Correlation network analysis of the allergic contact dermatitis (ACD) reactions identified 86 miRNAs as highly positively or negatively correlated with 1336 mRNAs (Figure 1F). In addition, 18 of the 86 highly correlated miRNAs were found to be differentially expressed, indicating that highly differentially expressed miRNAs contribute to transcriptomic changes during ACD. Moreover, identified mRNA targets were validated using the miRecords, miRTarBase and TarBase database, showing an overlap of 556 genes supporting the relevance of these targets (Figure 1G). Based on the 1336 mRNAs correlating with miRNA in ACD, enrichment analyses of the biological processes and pathway enrichments were performed and identified significant enrichment in processes of high relevance to ACD including T cell activation, cytokine signaling, and skin development (Figure S2).

Assuming that differentially expressed as well as highly correlated miRNAs are of biological relevance, we focused on the 18 ACD-related miRNAs (Figure S1B). Generating a miRNA-mRNA network with these miRNAs, we explored six of them with relevant functional enrichment (Figure 2A). Of these, miR-142-3p, miR-142-5p, miR-146b-5p and miR-155-5p were differentially expressed across all investigated allergens. Interestingly, only mRNA targets of miR-155-5p exhibit GO biological processes enrichment for axon

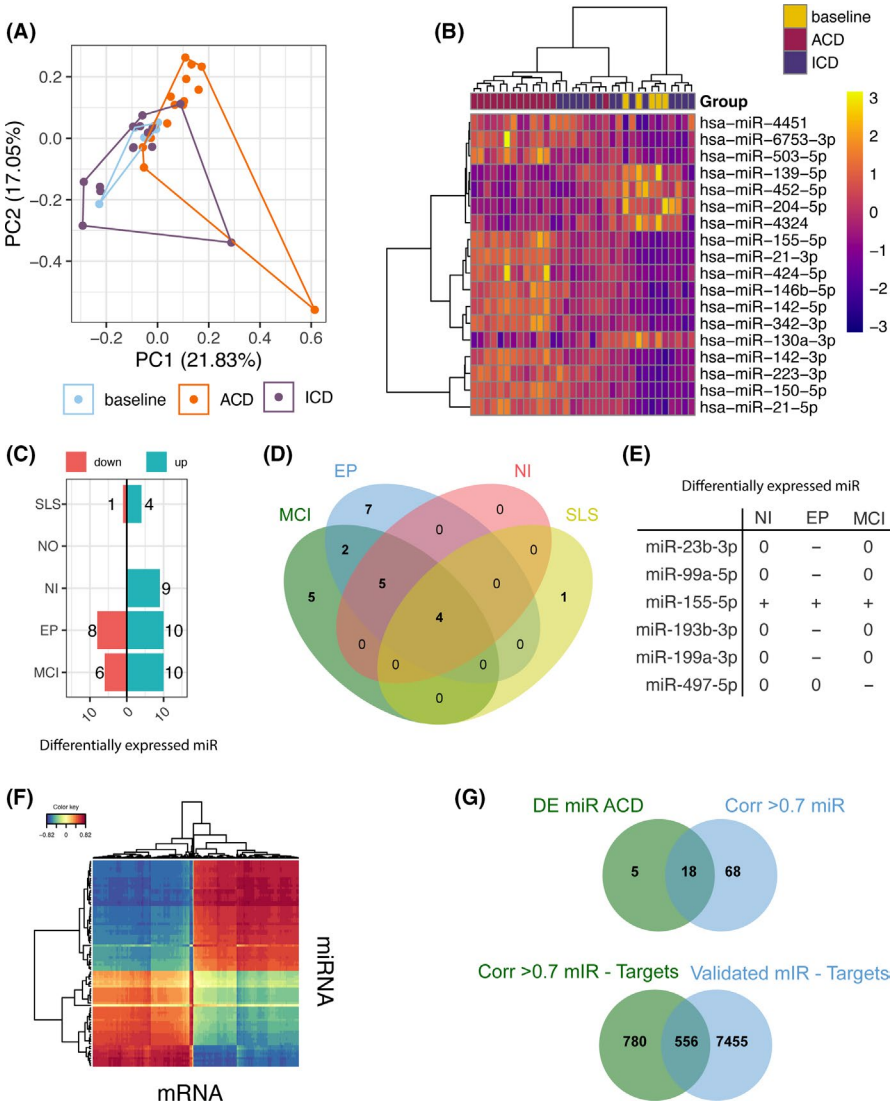

FIGURE 1 Allergic contact dermatitis and ICD induce different miRNA patterns. Principal component analysis (A) and hierarchical clustering (B) of miRNAome data. (C) Up- and down-regulated differentially expressed (DE) miRNAs. (D) Venn diagram of shared and unique miRNAs among the groups. (E) Highly relevant ACD-related miRNAs; up- (+), or down-regulated (-), non-DE (0). (F) Heat map displaying highly (>0.7) correlated miRNA–mRNA pairs. (G) Venn comparison demonstrating overlap between highly correlated and DE miRNAs (top), and highly correlated and previously validated miRNA-targets (bottom), respectively

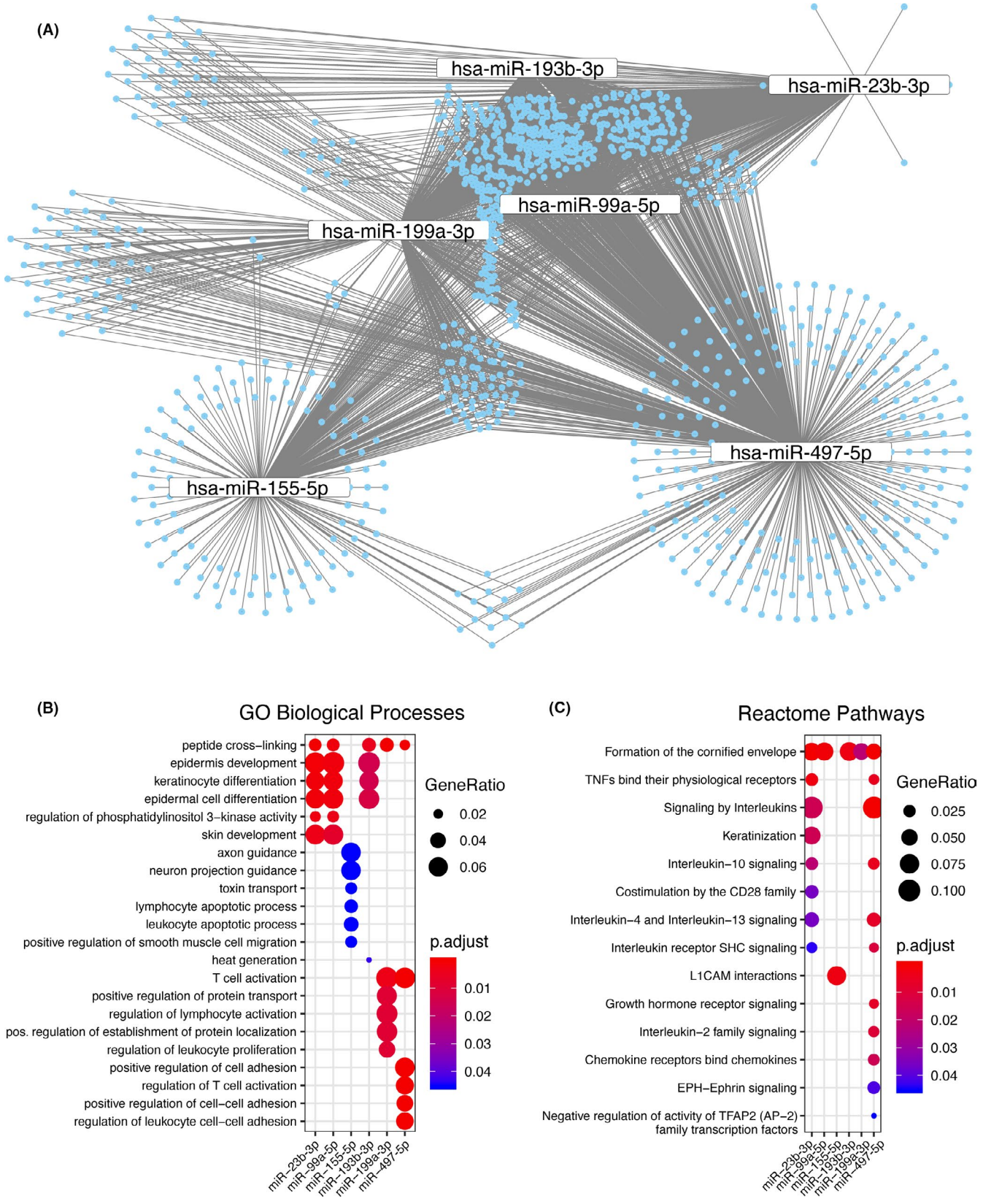

FIGURE 2 miRNA networks regulating mRNA during ACD. (A) Network of differentially expressed and top correlated miRNA-mRNA pairs in ACD. The network was visualized using Cytoscape. Gray lines illustrate correlation between the miRNA (white) and mRNA (blue dots). Enrichment analysis for GO biological processes (B) and reactome database (C) of highly correlated miRNA-mRNA target genes of ACD samples

guidance, smooth muscle cell migration and leukocyte/T cell apoptotic process (Figure 2B). Similarly, Reactome pathway analysis for the same mRNA targets revealed L1CAM interaction involvement (Figure 2C). miR-142-3p/5p have been described to be involved in ACD in both humans and mice,[3] as well as in other inflammatory skin disorders[1] and our data suggest that miR-142-3p/5p and miR-155-5p share some mRNA targets. Additionally, miR-155-5p appears to be a key regulator of the inflammatory skin response via the regulation of tight junction expression[4] as well as inflammation.[5]

Furthermore, we identified miRNAs which were uniquely regulated in MCI and EP encounter (Figure S1B). miR-497-5p was differentially expressed in MCI only, showing a high correlation with genes associated with T cell activation, cell-cell adhesion (Figure 2B) as well as cytokine and chemokine regulation pathways (Figure 2C). The function of miRNA-497-5p in human skin needs further elucidation; however, previous studies suggest a putative role in TGF-β-pathways via the regulation of SMAD3.[6] We identified SMAD3/SMAD4/SMAD5/SMAD7 as targets for miR-497-5p in our correlation experiments, underlining the importance of this miRNA during ACD.

Epoxy resin -induced allergic reactions regulated miR-23b-3p, miR-99a-5p, miR-193b-3p, and miR-199a-3p expression (Figure S1), which was not previously described in ACD. miR23b-3p and miR-99a-5p, appear to affect skin homeostasis and development in-vitro via TGIF1 and IGFR1, respectively.[7] Our findings underline the importance of those miRNAs since enrichment and pathway analysis revealed involvement in skin and epidermis development (Figure 2B,C). Lastly, miR-193b-3p and miR-199a-3p were described during oncogenic processes such as epidermal squamous cell carcinoma.[8] Both seems to interact with cell cycle genes controlling cellular proliferation. Our data pinpoint these proposed functions since both miRNAs show targets enriched for leukocyte proliferation and keratinocyte/epidermis differentiation. Taken together, our data suggest that the functional impact of the allergen-induced changes in the miRNAome on a cellular level may arise as a result of the interaction between several miRNAs regulating the same pathways. The fact that each miRNA targets hundreds of genes, and each gene is targeted by several miRNAs,[1] adds complexity to the interactions.

Contrary, initial correlation analysis of the irritant contact dermatitis (ICD) reactions revealed multiple correlations of miRNAs with lncRNAs (Figure S3). Since lncRNAs can act as mRNA regulators as well as "miRNA-sponges",[9] we created a miRNA-lncRNA-mRNA network using the same approach. We identified lnc-TRIM27-15 and lnc-TAF13-1 as central lncRNAs with their associated mRNA targets displaying enrichment for keratinocyte/epidermal differentiation and skin development (Figure S4). However, this computational model needs to be verified in further in-vitro studies.

In conclusion, we analyzed changes in the miRNA-mRNA expression in ACD and ICD and integrated our findings from the miRNAome with mRNA expression profiles from the same samples. Using this holistic approach, we found functional miRNA-mRNA networks linked to allergic and irritant responses in CD. Our study reveals a unique miRNA signature induced by ACD, with network construction of miRNA-mRNA pairs with enrichment of immunological responses including T cell activation and cytokine signaling.

To the best of our knowledge, this is the first study to integrate the miRNA and mRNA expression profiles in skin from sensitized subjects exposed to allergens and irritants, identifying known and novel miRNA-mRNA networks during CD (summarized in Figure S5). However, more studies are warranted for verification and further elucidation of the underlying regulatory mechanisms of ACD for the purpose of unraveling new therapeutic and diagnostic strategies.

KEYWORDS

allergic contact dermatitis, irritant contact dermatitis, miRNA, miRNA-mRNA integration

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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Paulina Werner[1]
Lukas Wisgrill[1,2]
Matilda Riskumäki[1]
Erja Jalonen[3]
Johanna Vendelin[4]
Sari Suomela[4]
Antti Lauerma[3]
Harri Alenius[1,5]
Nanna Fyhrquist[1,5]

[1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*
[2]*Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna, Austria*
[3]*Skin and Allergy Hospital, Helsinki University Hospital, Helsinki, Finland*
[4]*Finnish Institute of Occupational Health, Helsinki, Finland*
[5]*Department of Bacteriology and Immunology, Medicum, University of Helsinki, Helsinki, Finland*

Correspondence
Lukas Wisgrill, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden & Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna, Austria.
Email: Lukas.wisgrill@meduniwien.ac.at

ORCID

Paulina Werner https://orcid.org/0000-0002-8112-0252
Lukas Wisgrill https://orcid.org/0000-0001-9833-9499
Matilda Riskumäki https://orcid.org/0000-0002-1465-9055
Harri Alenius https://orcid.org/0000-0003-0106-8923
Nanna Fyhrquist https://orcid.org/0000-0002-5408-0005

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The H_4R is highly expressed on eosinophils from AD patients and IL-4 upregulates expression and function via the JAK/STAT pathway

To the Editor,

Histamine is involved in the pathological process of atopic dermatitis (AD) where it serves as mediator of inflammation and pruritus.[1] Histamine mediates its effects via four different G-protein coupled receptors (H_1R-H_4R), which are widely expressed on various immune cells and keratinocytes.[2] In recent years, many studies in particular focussed on the H_4R, which became a promising target in the therapy of AD.[1] It has been reported that eosinophils express a functional H_4R and that it is involved in chemotaxis, calcium influx, as well as in shape change.S1-S4 This study aimed to examine the expression pattern and regulation of histamine receptors on eosinophils under Th2 conditions and to investigate possible immunomodulatory functions especially of the H_4R. For this purpose, we isolated human eosinophils from peripheral blood via density centrifugation and negative bead selection (for details see Appendix S1). We found that the H_2R and the H_4R were regularly detectable at mRNA level (Figure 1), while the H_1R and H_3R were absent (data not shown). In former studies, we observed an upregulation of the H_4R under IL-4 treatment in $CD4^+$ T cells and during the differentiation from monocytes to monocyte-derived dendritic cells.[3,4] First, we stimulated eosinophils with Th2-associated cytokines, in particular IL-4 and IL-13 as well as with IL-3 and IL-5. All tested cytokines induced a significant upregulation of the H_4R, while only IL-4 and IL-3 induced an increase in H_2R expression (Figures 1A-F and S1). Subsequently, we focused on IL-4R signaling. For IL-4 and IL-13, upregulation of the H_4R expression was dose dependent (Figure 1D-F). Therefore, it appears that upregulation of the H_4R by these cytokines is a common mechanism in different cell types underlying the importance of IL-4R signaling in the regulation of H_4R expression. Intriguing, the H_2R was only upregulated via IL-4 stimulation and not via IL-13 (Figure 1A-C). While IL-4 signals through both the type 1 receptor composed of the IL-4Ra and common gamma chain and the type 2 receptor composed of the IL-4Ra and IL-13Ra, IL-13 mainly mediates its effects via the type 2 complex.S5 Thus, it seems that the H_4R can be regulated via both complexes, while the H_2R is only upregulated via the type 1 receptor complex.

Considering the most relevant downstream targets of the IL-4R, we found that the JAK/STAT pathway plays an important role in the H_4R upregulation. In contrast, we did not see effects via the MEK and AKT pathway (Figure 1G-I). Of note, the JAK1/2 inhibitor