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miRNA miR-17-92 cluster is differentially regulated in the imiquimod-treated skin but is not required for imiquimod-induced psoriasis-like dermatitis in mice

Dinghong Wu^{1,2,3}, Xinling Bi^{1,2}, Le Qu^{1,2}, Ling Han^{1,2,3}, Congcong Yin^{1,2}, Jingwen Deng^{1,2,3}, Zheng Dong⁴, Qing-Sheng Mi^{1,2,5,6,*}, and Li Zhou^{1,2,5,6,*}

¹Henry Ford Immunology Program, Henry Ford Health System, Detroit, MI

²Department of Dermatology, Henry Ford Health System, Detroit, MI

²Department of Dermatology, Guangdong provincial Hospital of Chinese Medicine, Guangzhou, P. R. China, 510120

⁴Department of Cellular Biology and Anatomy, Augusta University, Augusta, GA

⁵Department of Immunology and Microbiology, Wayne State University, Detroit, MI, USA

⁶Department of Internal Medicine, Henry Ford Health System, Detroit, MI

Abstract

MicroRNAs (miRNAs) play very important roles in the control of immune cell and keratinocyte development and function and are implicated in skin inflammatory diseases, including psoriasis. MiRNA miR-17-92 was reported to promote the differentiation of Th1 and Th17 cells, and to regulate cell proliferation and apoptosis. Here we showed that imiquimod (IMQ) differentially regulates the expression of miR-17-92 cluster in the mouse skin, upregulating miR-17 and miR-19 families and downregulating miR-92. To investigate if miR-17-92 cluster is functionally involved in the psoriasis, we have generated three mutant mice with specific deletion or overexpression of miR-17-92 cluster in keratinocytes, or with deletion of miR-17-92 cluster in T cells. Interestingly, deletion or overexpression of miR-17-92 cluster in keratinocytes, or deletion of miR-17-92 in T cells did not significantly affect IMQ-induced psoriasis-like dermatitis development in the mutant mice compared to wild-type littermates. Thus, miRNA miR-17-92 cluster may not be a key factor regulating imiquimod-induced psoriasis-like dermatitis.

Keywords

miRNAs; psoriasis; T cells; imiquimod; knockout; knockin

*To whom correspondence should be addressed: Dr. Qing-Sheng Mi, Henry Ford Immunology Program, Department of Dermatology and Department of Internal Medicine, Henry Ford Hospital, 1 Ford Place, Detroit, MI, United States. qmi1@hfhs.org. Or Li Zhou, Henry Ford Immunology Program, Department of Dermatology and Department of Internal Medicine, Henry Ford Hospital, 1 Ford Place, Detroit, MI, United States. lzhou1@hfhs.org.

Conflict of interests

The authors have declared no conflicting interests.

Background

Psoriasis is an immune-mediated inflammatory skin disease, characterized by keratinocyte hyperproliferation and altered differentiation, the presence of an inflammatory cell infiltrate and neovascularization. Both Th1 and Th17 cells contribute to psoriasis development. MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate gene expression through either translational repression or mRNA degradation. Using a mouse model with the deletion of Dicer, an enzyme required for the processing of mature and functional miRNAs, studies from our group and others indicated that lack of miRNAs by deletion of Dicer dramatically interrupts immune system and skin development and function (1). Accumulated studies have showed that specific miRNAs are key regulators in the control of immune cell and keratinocyte development and function and implicated in skin inflammatory diseases, including psoriasis (2–4). MiRNA miR-17-92 cluster, located on human chromosome 13q31 and on murine chromosome 14, is one of the polycistronic miRNA clusters, and contains 6 individual miRNAs: the miR-17 family (miR-17, miR-20, and miR-18a), the miR-19 family (miR-19a and miR-19b), and miR-92a. miR-17-92 is involved in a wide variety of malignancies and regulates cell proliferation and apoptosis (5)(S1). miR-17-92 cluster promotes proliferation and inhibits differentiation of keratinocytes during embryogenesis. More recently, miR-17-92 was also reported to promote the differentiation of Th17 cells and Th1 cells (6, 7). Most interestingly, previous studies showed the abnormal expression of miR-17-92 in human psoriasis (2–4). However, it remains unclear whether miR-17-92 is functionally involved in the pathogenesis of psoriasis.

Question addressed

Using keratinocytes- and T cells- specific miR-17-92 knockout (KO) mice and keratinocytes-specific miR-17-92 knockin (KI) mice to address the role of miR-17-92 in the pathogenesis of psoriasis.

Experimental design

MiR-17-92loxp and miR-17-92KI mice were described previously (5, 8)(S1). Keratinocytes-specific K14-miR-17-92KO mice and K14-miR-17-92KI mice were generated by crossing miR-17-92loxp mice or miR-17-92KI mice with the K14-Cre mice; T cells-specific CD4-miR-17-92KO mice were generated by crossing miR-17-92loxp mice with CD4-Cre mice. The mouse genotyping was based on previous report (S1). Mice at 8 weeks of age were shaved and then the hair on the back were removed with Nair, and received daily, for 5 consecutive days, topical applications of 60 mg imiquimod (IMQ) (Sichuan Med-shine pharmaceutical Co, China). Skin lesions were scored based on the mouse psoriasis severity index (PASI) score described recently (9) to evaluate the back skin inflammatory and were collected for HE staining at day 6 after IMQ treatment. Skin from C57BL6 mice treated with IMQ at day 6 were collected to test the expression of miR-17-92 cluster by TaqMan RT-PCR. The mice were raised in a specific pathogen-free barrier unit. All experimental procedures were approved by the Institutional of Animal Care and Use Committee. ANOVA with Bonferroni test, or paired and unpaired Student's t test were applied for PASI score,

epidermal layers and gene expression between mutant and WT mice or comparing IMQ treated versus vehicle-treated mice.

Results

To investigate if miR-17-92 is involved in IMQ-mediated psoriasis, we first examined the expression of miR-17, miR-18a, miR19a, miR-19b, miR-20a and miR-92a in the skin treated with IMQ for 5 days in C57BL6 mice. As shown in Fig 1, the expression of miR-17, miR19a, miR-19b and miR-20a were significantly up-regulated in IMQ-treated skin compared to the mice treated with the vehicle, while the expression of miR-18a was unchanged. However, the expression of miR-92a was down-regulated. Interestingly, upregulated miR-17 and miR-20a were reported in the lesions of human psoriasis (2–4), suggesting that dysregulated miR-17-92 cluster may contribute to psoriasis development. To uncover the function of miR-17-92 in psoriasis, we first tested if specific deletion of miR-17-92 in keratinocytes can prevent from IMQ-induced psoriasis. As shown in Fig 2a, the PASI score appeared comparable between K14-miR-17-92KO mice, in which keratinocytes specifically lack miR-17-92 (Fig. S1A), and littermate control, suggesting that IMQ-induced upregulation of miR-17, miR19a, miR-19b and miR-20a may not contribute to psoriasis development. To further investigate if upregulating miR-17-92, especially for miR-92 in keratinocytes can regulate IMQ-mediated psoriasis, K14-miR-17-92KI mice, in which keratinocytes overexpress miR-17-92 (Fig. S1B), were used. As shown in Fig 2b, there was no significant difference in PASI scores between K14-miR-17-92KI and littermate control either, suggesting IMQ-mediated downregulation of miR-92 may not be involved in psoriasis development. Thus, IMQ-induced differential miR-17-92 expression in keratinocytes may not be key factors involved in psoriasis development. Given both Th1 and Th17 cells are involved in psoriasis development and miR-17-92 promotes Th1 and Th17 cell differentiation, we next asked if deletion of miR-17-92 in T cells can prevent from IMQ-induced psoriasis development. As shown in Fig 2c, surprisingly, there was no significant difference in PASI scores between CD4-miR-17-92KO and littermate control. Consistent with comparable clinical scores between miR-17-92 mutant mice and WT mice, the histological analyses of epidermal hyperplasia at day 6 after IMQ treatment did not reveal the significant differences between miR-17-92 mutant mice and WT mice (Fig 2d–f).

Conclusions

Our results suggest that IMQ differentially regulates the expression of miR-17-92 cluster in skin, upregulating miR-17 and miR-19 families and downregulating miR-92. However, deletion or overexpression of miR-17-92 cluster in keratinocytes, or deletion of miR-17-92 in T cells did not significantly affect IMQ-mediated psoriasis. Thus, overall, current findings suggest that miR-17-92 cluster is not required for imiquimod-induced psoriasis-like dermatitis. Given the potential roles of miR-17-92 cluster in promoting proliferation of keratinocytes and enhancing Th1 and Th17 differentiation, the results from our study were unexpected. One of explanations is that miR-17 and miR-19 families and miR-92 in miR-17-92 cluster may have different function in keratinocytes and T cells in IMQ-induced psoriasis, and these miRNAs may counter-regulate each other during psoriasis development. Thus, deletion or overexpression of whole miR-17-92 cluster in keratinocytes or T cells may

not really reflect the individual miRNA function. Individual miRNA function needs to be further explored in the individual cell-specific mutant mice, which are currently under the investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

The expression of miR-17-92 in IMQ-treated skin lesion. C57BL6 mice were treated daily with 60mg IMQ cream or vehicle control on the shaved back skin for continuously 5 days. Skin lesion were collected at day 6, and the expression of miR17-92 cluster were tested by RT-PCR. N= 3 mice, * P<0.05, **P<0.01, ***P<0.001

Fig. 2.

IMQ-induced psoriasis-like skin inflammation in miR-17-92 mutant mice. **a–c**, miR-17 mutant mice and WT littermates were treated daily with 60mg IMQ cream on the shaved back skin. PASI score (erythema, scaling and induration) of the back skin were scored daily, symbols indicate mean PASI score $\pm$ SD. **d–f**, the skin lesions were collected at day 6 after IMQ treatment for HE staining and number of epidermal cell layers were counted per high power field. (a and d), K14-miR17KO mice and WT Mice; (b and e), K14-miR-17-92KI mice and WT Mice; (c and f), CD4-miR-17-92KO mice and WT Mice. $N=9-15$, $P > 0.5$.