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# Evidence on the direct correlation between miR-31 and IL-22 axis in IMQ-induced psoriasis

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Psoriatic skin is characterized by a deregulated profile of miRNAs that are contributing in disease development. In this study, we focus on miR-31, one of the upregulated miRNAs known to promote keratinocytes proliferation and migration. Moreover, miR-31 expression induction was dependent on a large panel of cytokines including IL-22. Here, we aimed at investigating the relationship between miR-31-5p and IL-22 axis; and by searching novel molecular target for miR-31-5p in keratinocytes. Our data identify a direct correlation between miR-31-5p and IL-22 in psoriasis with Pwp1 as new potential target. These findings confirm the important role of miR-31 in psoriasis onset and provide a basis for further investigations in miRNAs field in context of skin diseases.

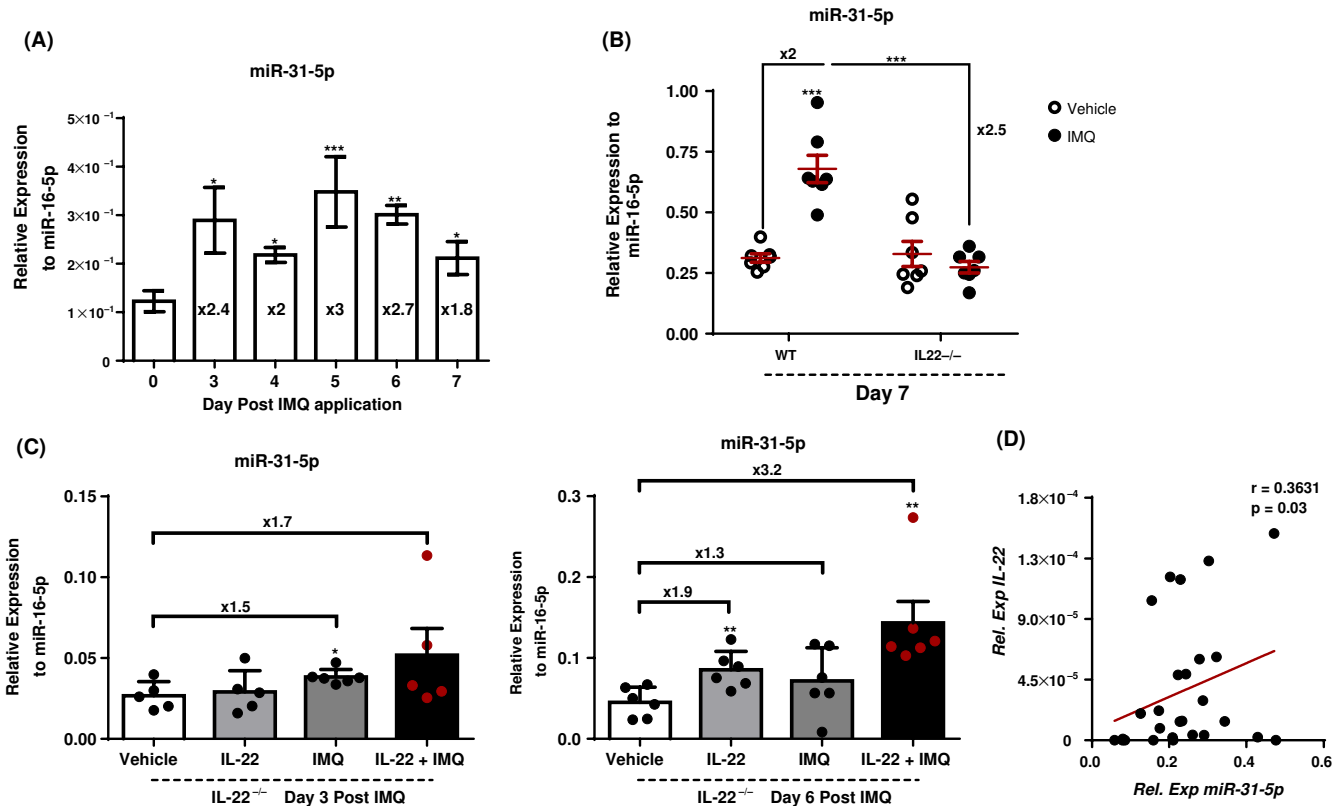
## 1 | BACKGROUND

Although psoriasis generally does not affect patients' survival, it has a strong psychosocial well-being impact, interfering with patients' quality of life by negatively altering the physical and emotional behaviour of affected patients.<sup>[1]</sup> It is true that the clinical and scientific progresses with the biologics treatment has revolutionized and improved the disease perception, management and patient's care. Nevertheless, psoriasis remains an incurable disease till now. Therefore, novel advances in comprehending the molecular settings interfering with the immunological occasions to provide new insights, novel biomarkers and new treatments strategies are necessary. Emerging studies showed a specific psoriatic microRNAs signature where miR-31 is one of the most upregulated microRNAs in psoriatic skin.<sup>[2,3]</sup> Furthermore, different studies demonstrated its ability to promote wound healing and to modulate inflammatory mediators production in keratinocytes (KCs).<sup>[3-5]</sup> More interestingly, the genetic deficiency of miR-31 in KCs inhibits their hyperproliferation

and reduces the disease severity in psoriasis mouse models.<sup>[6]</sup> The imbalanced KCs hyperproliferation and differentiation that manifest as thick red irritated skin is caused mainly by a complex crosstalk between epithelial cells and immunocytes under an enriched inflammatory environment.<sup>[7-9]</sup> Among the upregulated inflammatory mediators, IL-22 an IL-10 cytokine is a well known and described to induce KCs hyperproliferation.<sup>[10]</sup> Besides IL-22-deficient mice were shown to develop a less severe form of psoriasis.<sup>[11]</sup> Thus, the common proliferative activity of these upregulated actors (miR-31 and IL-22) drove us to investigate their potential correlation in psoriasis context.

## 2 | QUESTIONS ADDRESSED

This study aimed to address the relevance of miR-31 in IMQ mice model, to define its involvement in the pathogenesis development and to verify its dependency on IL-22 axis.



**FIGURE 1** miR-31 induction in psoriasis is IL-22 dependent. The expression pattern of miR-31-5p was evaluated in psoriatic skin from wild type (WT) mice treated with 60 mg of IMQ (A). Relative expression of miR-31-5p to miR-16-5p in WT vs IL-22<sup>-/-</sup> non-treated vaseline (vehicle) mice or IMQ-induced psoriasisform mice at day 7 (B). IL-22<sup>-/-</sup> mice were injected subcutaneously with 100 ng of IL-22 or equivalent of physiological serum 1 d before pathogenesis induction and up to 7 d, miR-31 relative expression was assessed on day 3 and day 6 post IMQ application (C). Spearman correlation between relative expression of miR-31-5p and IL-22 was established in 27 psoriatic skin from WT mice at day 1 and from day 3 to 7 in IMQ model (D). Two-way ANOVA: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Data are from two independent experiments with at least five mice per group (fold change, mean and SEM)

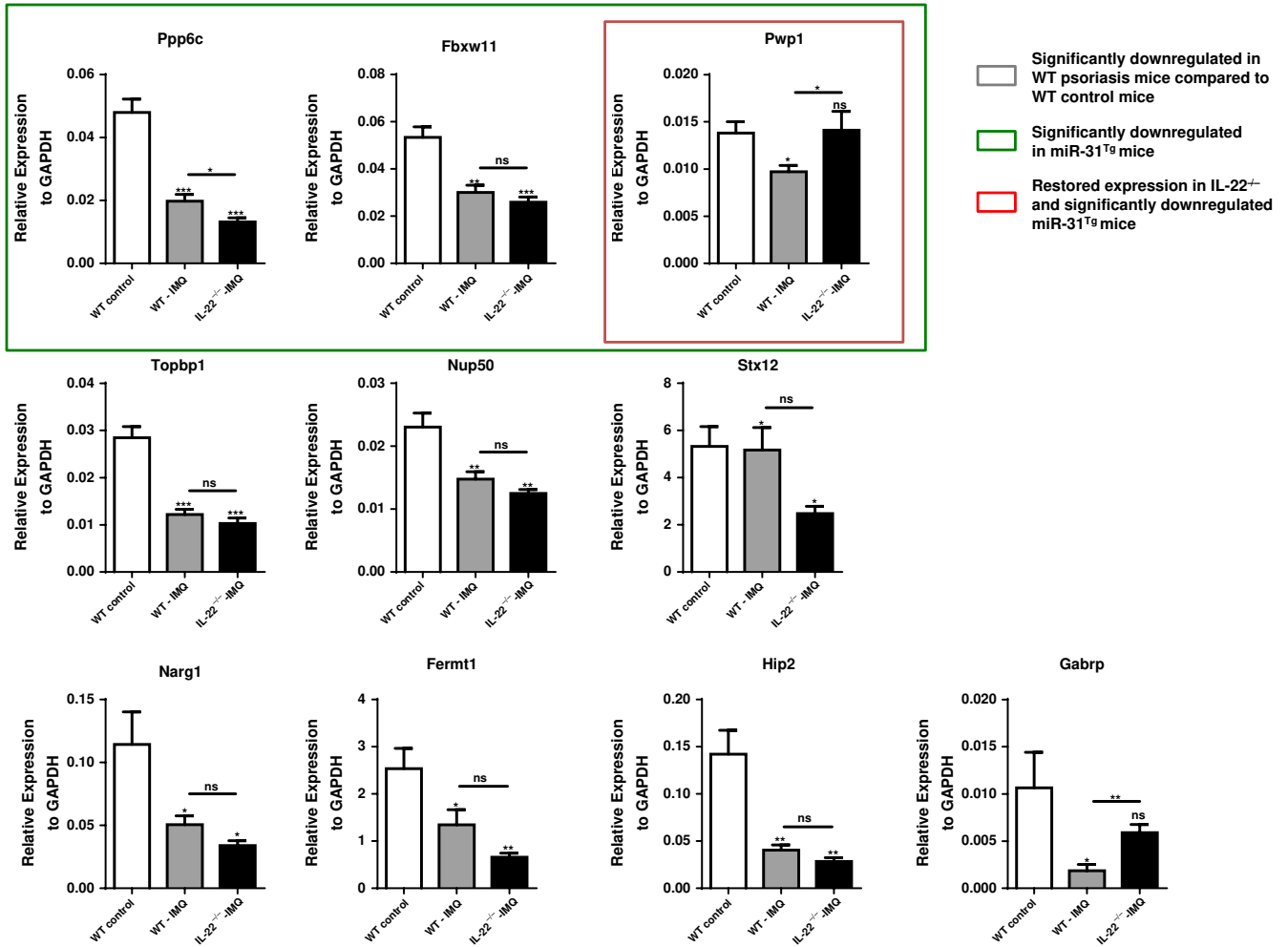
### 3 | EXPERIMENTAL DESIGN

See Data S1.

### 4 | RESULTS

To investigate miR-31-5p implication and regulation during psoriasis development, we used imiquimod (IMQ)-induced psoriasisform mice model (Figures S1 and S2). First, we followed miR-31-5p expression pattern throughout the pathogenesis (Figure 1). Wild-type (WT) mice were treated for six consecutive days with 60 mg of IMQ on the shaved back. Then, the relative expression of miR-31-5p was measured by RT q PCR at day 1 and from day 3 to 7, which correspond to psoriasis phenotypes onset (Figures S1 and S2). The obtained results showed an upregulation of miR-31-5p expression from day 3 and all over the pathogenesis progression. This upregulation varies significantly between 1.8- and 2.9-fold increase compared to day 1, yet this variation along the disease evolution is not significant. These findings show the active implication of miR-31-5p at all stages of the pathology evolution (Figure 1A). Since miR-31 and IL-22 have been

demonstrated to have a common hyperproliferative role and that miR-31-5p expression induction in primary KCs was IL-22 dependent,<sup>[6]</sup> we sought to examine the correlation of miR-31-5p and IL-22 axis in psoriasis. For this, we used IL-22 deficient mice (IL-22<sup>-/-</sup>) to compare the expression profile of miR-31-5p with that of WT mice in IMQ model (Figure 1B). At steady state, miR-31-5p relative expression was similar between both genotypes. As shown previously, miR-31-5p was upregulated significantly up to 2-fold in IMQ-treated WT mice compared to WT control. On the contrary, IL-22<sup>-/-</sup> mice treated with IMQ were unable to regulate miR-31-5p as they express the same level of miR-31-5p as that of IL-22<sup>-/-</sup> mice control. Interestingly, psoriatic IL-22<sup>-/-</sup> mice had 2.5 times less miR-31-5p than psoriatic WT (Figure 1B). These results suggest a direct correlation between miR-31-5p and IL-22 pathway. Nevertheless, the mechanism by which IL-22 can regulate miR-31 in psoriasis can be discussed. miR-31 promoter contains NF- $\kappa$ B (p65) and STAT3 binding sites.<sup>[12]</sup> In wound healing models, miR-31 expression was shown to be dependent of NF- $\kappa$ B and STAT3 activities at the inflammatory phase.<sup>[5]</sup> The induction of miR-31 expression mediated by NF- $\kappa$ B activation was shown by Yan et al<sup>[6]</sup> to be correlated with a large panel of cytokines IL-1 $\alpha$ , IL-6, IL-17A, TNF- $\alpha$ , IFN- $\gamma$  but not IL-22 in KCs.



**FIGURE 2** Identification of IL-22 dependent miR-31 potential gene target. mRNA analysis profile of 20 published predicted miR-31 targets were done on RNA extracted from WT control mice, WT IMQ treated mice and IL-22<sup>-/-</sup> IMQ treated mice at day 7. Significantly downregulated targets in WT IMQ compared to WT control are presented here (grey square). The commonly downregulated targets between WT-IMQ and miR-31Tg mice are highlighted in the green rectangle. The selected target that is upregulated in IL-22<sup>-/-</sup> and downregulated in miR-31Tg mice is highlighted in red. The unselected targets are shown in Figure S4. Two-way ANOVA: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. Data are from three independent experiments with at least four mice per group (fold change, mean and SEM)

These results suggest that STAT3, one of the major transcription factors activated downstream to IL-22 stimulation could be responsible of miR-31 induction in KCs. Thus, miR-31 induction by IL-22 could be dependent of STAT3. Therefore, to confirm IL-22/miR-31 connection, we treated subcutaneously IL-22<sup>-/-</sup> mice with 100 ng IL-22 or the equivalent of physiological serum 1-day prior and all along the 6 days of IMQ or vehicle application (Figure 1C). We chose day 3 and day 6; two time points postdisease development to explore miR-31-5p relative expression in those different conditions. At day 3, the variation was very low between vehicle, IL-22, IMQ-treated IL-22<sup>-/-</sup> and mice co-treated with IL-22 and IMQ. At day 6, this increase became significant and reached over 3-fold upregulation. Note that IL-22 treated IL-22<sup>-/-</sup> mice upregulated significantly miR-31-5p with almost twice the expression of the control. These observations provide arguments on the correlation between miR-31-5p and IL-22 axis. The positive spearman correlation reinforces this conclusion (Figure 1D).

Then, we took advantage of the published 20 potential targets of miR-31-5p predicted from IMQ-treated miR-31 transgenic mice (miR-31<sup>Tg</sup>) (Table S1) [6] to identify a common hit between miR-31-5p and IL-22. Briefly, Yan et al [6] used four bioinformatics tools (TargetScan, miRDB, miRWalk and RNA22) to predict the potential miR-31-5p target genes. Consequently, the analysis revealed 20 common hits between the different bioinformatics tools that were considered as the putative target mRNAs of miR-31. We assayed 20 mRNAs in WT control, IMQ-treated WT and IMQ-treated IL-22<sup>-/-</sup> mice by RT-qPCR (Figures 2 and S4). Since miRNAs-silencing activity occurs *via* transcript degradation or translational arrest, the target selection strategy was based on 2 groups of interest.<sup>[13-15]</sup> The strategy is illustrated in Figure S3. First, we selected significantly downregulated mRNA in WT psoriasis mice having increased miR-31 expression compared to healthy ones (Narg1, Fbxw11, Nup50, Gabrp, Pwp1, Topbp1, Ppp6c, Nip2 and Fermt1) (Figure 2). From the selected mRNAs,

we kept mRNAs (Pwp1, Ppp6c, Fbxw11) that were significantly downregulated in miR-31<sup>Tg</sup> (Figure 2). Then, we spotted the upregulated mRNAs in IL-22<sup>-/-</sup> compared to WT psoriatic mice. Only Pwp1<sup>[16]</sup> presented the same expression level as WT control and significantly upregulated expression compared to WT psoriatic mice (Figure 2). Hence, these observations speculate that Pwp1 is more likely to be one miR-31 target in an IL-22 dependent manner.

## 5 | CONCLUSION

In summary, this study shows the upregulation of miR-31-5p throughout the psoriasis immunopathogenesis and for the first time, we evidenced a concrete in vivo correlation between miR-31 upregulation and IL-22 signalling pointing out the complex crosstalk between immune cells, inflammatory mediators and miRNAs responsible for psoriasis development. Finally, we predict Pwp1 (periodic tryptophan protein 1 associated with histone modification) as a potential novel mRNA target for miR-31-5p in KCs. This hit was shown to be a regulator of the JAK/STAT3 signalling pathway. Shen and colleagues reported that the knockdown of Pwp1 promotes STAT3 upregulation.<sup>[17,18]</sup> This result strengthens the fact that miR31 by targeting of Pwp1 mRNA will result in STAT3 upregulation in an IL-22 dependent manner. Altogether, these findings emphasize the important role of miR-31 in psoriasis and propose a new molecular hit that needs to be considered with the immune occasions for psoriasis and inflammatory skin disorders.

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## CONFLICT OF INTEREST

The authors have declared no conflicting interests.

## AUTHOR CONTRIBUTIONS

Funding acquisition and supervision: CP; Experimental design and performance: FA.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Psoriasis-like lesions development after IMQ topical application in wild-type (WT) vs IL-22<sup>-/-</sup> mice. Mice between 8 and 10 wk of age were shaved on the back skin and treated for 6 consecutive days by 60 mg of imiquimod (IMQ) topical application or the equivalent with Vaseline (Vehicle) (a). The weight fluctuation indicative of IMQ psoriasiform development was assessed all over the pathogenesis (b). IL-22<sup>-/-</sup> mice showed reduced psoriatic lesions compared to WT mice throughout IMQ application (c). H/E staining of back skin in the different groups at day 7 (d).

**Figure S2.** The kinetics of IL-22 relative expression to GAPDH in imiquimod induced psoriasis in wild type mice.

**Figure S3.** The strategy illustrating IL-22 dependent miR-31-5p targets selection.

**Figure S4.** The screening of IL-22 dependent miR-31 potential gene target. mRNA analysis profile of 20 published predicted miR-31 targets were done on RNA extracted from WT control mice, WT IMQ treated mice and IL-22<sup>-/-</sup> IMQ treated mice at day 7. Only the unselected targets are shown here. Two-way ANOVA: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Data are from three

independent experiments with at least four mice per group (fold change, mean and SEM).

**Data S1.** Function description of 20 predicted miR-31-5p targets (6).