MCPIP1 RNase is aberrantly distributed in psoriatic epidermis and rapidly induced by IL-17A

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Introduction

IL-17A-induced genes constitute clinically validated targets for psoriasis due to the elevated anti-psoriatic activity of biologics targeting the IL-17/IL-17R pathway. As IL-17RA is mostly distributed in differentiated suprabasal keratinocytes, it has been suggested that IL-17 modulate gene expression preferentially in this compartment. *ZC3H12A* gene encoding the RNase MCPIP1 is upregulated in psoriatic skin and reduced to normal levels after anti-IL-17A/IL-17AR neutralizing treatments and it has been included within the most upregulated transcripts in the IL-17A-induced signature in keratinocytes. However, there are no further studies characterizing its expression or function in psoriatic skin. In this study, we have investigated its distribution in psoriasis lesions and how IL-17A could be modulating its expression.

Materials and methods

Normal human epidermal keratinocytes were cultured until confluence and activated by recombinant human IL-17A or IFN-γ, followed by RNA extraction at indicated time points. Human skin biopsies were fixed in 4% formaldehyde and embedded in paraffin. Tissue sections were stained with H&E or were deparaffinized for immunostaining. For flow cytometry assays, epidermal cell suspensions were isolated from skin punch biopsies from 5 psoriatic and 3 healthy donors. 70,000 epidermal cells were stimulated at a final concentration of 10 and 50 ng ml^{-1} for 4 hours and stained for superficial CD29 β-integrin and for intracellular cytoplasmic KRT10 and MCPIP1. For silencing, HaCaT cells were transfected with a plasmid expressing shRNA sequence towards *ZC3H12A* mRNA and as a control of silencing we used plasmid carrying unspecific scrambled sequence.

Results

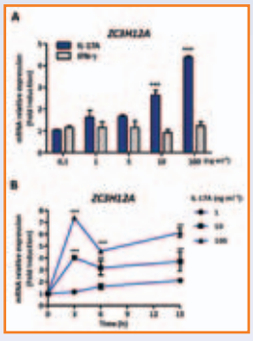

Figure 1. IL-17A, but not IFN-γ, induces upregulation of *ZC3H12A* mRNA in keratinocytes at early and late time points. (a) Keratinocytes were cultured and stimulated at confluence with different doses of IL-17A or IFN-γ. *ZC3H12A* mRNA expression was evaluated at 15h after treatment (n=2). IL-17A-treated keratinocytes showed a dose dependent induction of *ZC3H12A* gene expression, while no effect was observed upon stimulation with IFN-γ **(b)** mRNA from IL-17A (1, 10 and 100 ng ml^{-1}) stimulated keratinocytes was extracted at different early and late time points for *ZC3H12A* gene expression analysis. *ZC3H12A* upregulation was most efficient after 3 hours of treatment with high doses of IL-17A, although lower doses of this cytokine resulted in only slight induction after 6 and 15 hours. Bar and line charts show means with standard-deviation error bars.

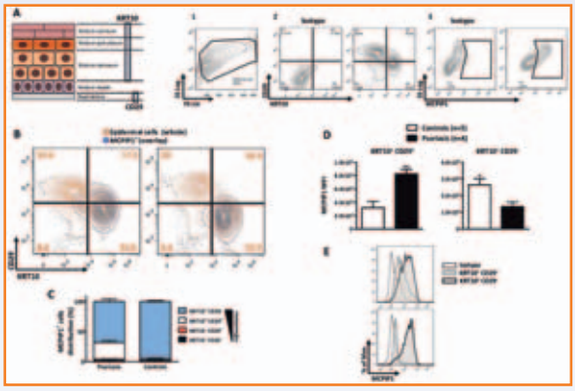

Figure 3. Abnormal MCPIP1 expression in psoriasis skin is distributed in high differentiating and suprabasal rapid-cycling epidermal population subsets. (a) Left illustration shows the distribution of suprabasal keratin marker KRT10 and the basal β-integrin CD29 in normal epidermis. 1 to 3 images show the gating strategy of flow cytometry data from one representative psoriatic sample with triple staining against CD29, KRT10 and MCPIP1. Image 3 shows MCPIP1 detection in KRT10$^+$CD29$^+$ quadrant of image 2. **(b)** Overlay of MCPIP1$^+$ cells (blue contour) over whole epidermal cells (orange contour) acquired on a KRT10 vs CD29 quadrant graph (bold numbers represent percentages of each quadrant population from whole epidermal population). One representative psoriasis and healthy samples are shown. **(c)** MCPIP1$^+$ cells distribution in epidermal subsets of psoriasis (n=4) and controls (n=3) samples. In psoriatic samples, MCPIP1 was additionally distributed through the suprabasal compartment coexpressing KRT10 and CD29, which corresponds to a rapid cycling proliferating cell population, while in controls it was almost exclusively confined to high differentiated KRT10$^+$CD29- cells. **(d)** MFIs for MCPIP1 expression in KRT10$^+$CD29$^+$ and KRT10$^+$CD29- cells, represented by histograms of one representative psoriatic and control sample **(e)**. KRT10$^+$CD29$^-$ population in controls was higher than in psoriasis epidermis, while KRT10$^+$CD29$^+$ cells were more intensively stained in psoriasis epidermis than in controls. Data are shown as mean ± SEM.

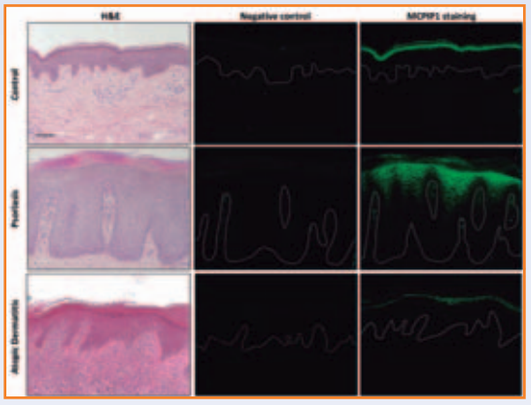

Figure 2. MCPIP1 is aberrantly expressed in suprabasal psoriatic epidermis. MCPIP1 localization by immunohistochemistry (IHC) and visualized by fluorescence microscopy. IHC staining showed a preferential expression in the epidermis and a wide localization and at high levels throughout suprabasal keratinocytes, especially beneath the apical side of the cells. Conversely, in control healthy skin this was limited to the upper granular layer, similarly to what was seen in samples from atopic dermatitis lesions Left images show serial sections in H&E staining. Scale bar = 100 μm.

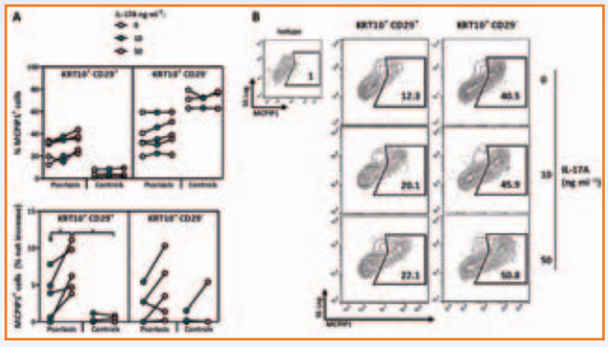

Figure 4. MCPIP1 is rapidly upregulated in differentiating epidermal cells by IL-17A. (a) Epidermal cell suspensions were cultured for 4 hours with or without IL-17A (0, 10 or 50 ng ml^{-1}), and were analyzed by flow cytometry. **(a)** Percentage of MCPIP1$^+$ cells (upper graph) and respective net increments (lower graph) were calculated subtracting basal MCPIP1 percentage expression, and were compared within the same individuals (paired-test) or with control values at same IL-17A concentration (t-test). We found a significant dose dependent increment of percentage of MCPIP1$^+$ cells in KRT10+ population, especially within KRT10$^+$CD29$^+$ subset in psoriasis. **(b)** MCPIP1 induction in KRT10$^+$ subsets in one representative psoriatic sample after 4 hours of IL-17A activation. Bold numbers indicate percentage of gated MCPIP1$^+$ cells.

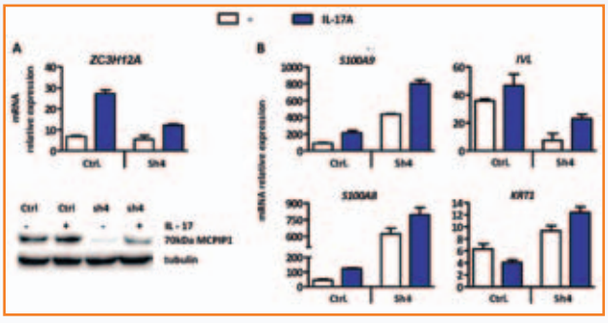

Figure 5. Silencing of *ZC3H12A*/MCPIP1 affects expression of transcripts present in the suprabasal epidermal cellular layer. (a) MCPIP1-silencing in HaCat keratinocytes by lentiviral plasmid transfection was verified at mRNA (qRT-PCR) and protein (western blot) levels after selection of positively-transduced cells which were stimulated or not with IL-17A for 15h (10ng/300μl). **(b)** Some psoriasis-associated genes were checked in the same samples. S100a family members *S100A8* and *S100A9*, at both basal levels and after IL-17A stimulation, were upregulated in HaCaT silenced cells. Interestingly, early-differentiation marker *IVL*, which is upregulated in psoriasis, was downregulated within *ZC3H12A* silenced cells. On the contrary, *KRT1* gene, which maintains skin integrity and is usually reduced in psoriasis, was increased in silenced conditions

Conclusion

These results show that MCPIP1 is aberrantly expressed in psoriatic differentiated epidermal layers and may be potentially involved in altered differentiation through an IL-17A-dependent induction. Further studies should provide new insights about the relevance of this RNase in the modulation of other genes, especially in IL-17A-regulated signatures associated to altered differentiation and cornification in psoriatic epidermis.

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