ZC3H12A encodes a ribonuclease highly expressed in psoriasis

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Introduction

ZC3H12A encodes a ribonuclease that is involved in mRNA and miRNA metabolism, but its expression and potential role in psoriasis has not been evaluated. In this study we have characterized its gene and protein expression using a previously described *ex vivo* model of psoriasis as well as other *in vitro/in vivo* approaches.

Material and methods

For *in vitro* studies, normal human keratinocytes (NHKs) were incubated with 1/10 diluted conditioned coculture supernatants or with recombinant cytokines. Mouse model of Imiquimod (IMQ)-induced psoriasis-like inflammation and human skin biopsies were used for in vivo assays. mRNA and protein expression were measured by qPCR and western blot, respectively, while the skin protein expression pattern was assessed by immunohistochemistry (IHC).

Results

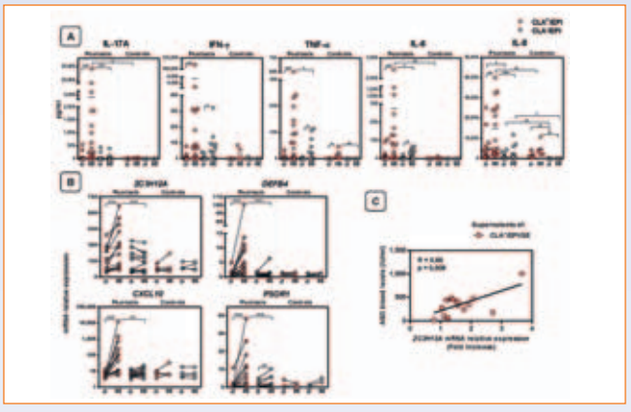

FIGURE 1: Supernatants from *Streptococcus*-activated cocultures of CLA$^+$ T cells seeded with lesional autologous epidermal cells induce ZC3H12A mRNA expression in NHKs. **(A)** Activation of cocultures of circulating CLA$^+$ memory T cells (CLA$^+$) with autologous epidermal cells (EPI) through a streptococcal extract (SE) resulted in the production of proinflammatory cytokines such as IL-17A, IFN-γ, TNF-μ, IL-6 and IL-8, which are important mediators in psoriatic lesions (Psoriasis n=21; Controls n=5). **(B)** NHKs stimulated with conditioned supernatants from SE-activated CLA$^+$/EPI cocultures showed upregulation of ZC3H12A mRNA expression compared with supernatants from coculture conditions with CLA$^-$ T cells (CLA$^-$) or without SE stimulation (Ø), as well as other genes involved in psoriasis (Psoriasis n=13; Controls n=5). Moreover, net ZC3H12A mRNA upregulation by SE-activated CLA$^+$/EPI coculture supernatants correlates with anti-streptolysin O blood levels **(C)**. Each dot represents one psoriatic or control patient.

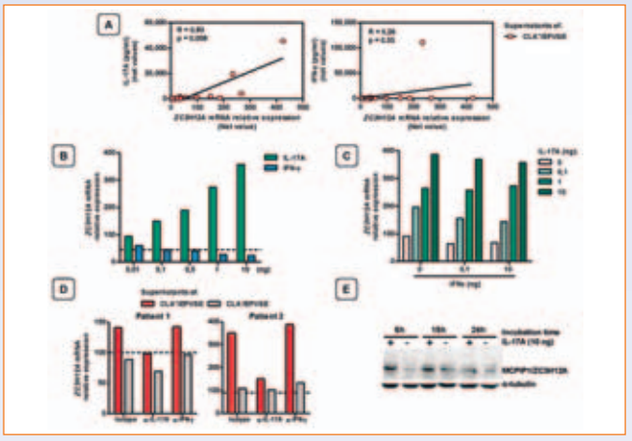

FIGURE 2: ZC3H12A gene and protein expression is induced by IL-17A. **(A)** Net IL-17A amounts from SE-activated CLA$^+$/EPI coculture supernatants, but not IFN-γ, correlate with ZC3H12A mRNA expression levels induced in NHKs. Activation of NHKs with human recombinant IL-17A and/or IFN-γ reveals that mRNA upregulation of ZC3H12A is IL-17A dependent **(B, C)**, which is supported by blocking antibody assays in our coculture supernatants **(D)**. Furthermore, HaCat cells stimulated with IL-17A showed upregulation of ZC3H12A protein level **(E)**. Dashed lines indicate basal expression. Final stimulation volume= 300 μl. Net values correspond to subtracting the basal cytokine production (Ø) from SE-activated condition (SE).

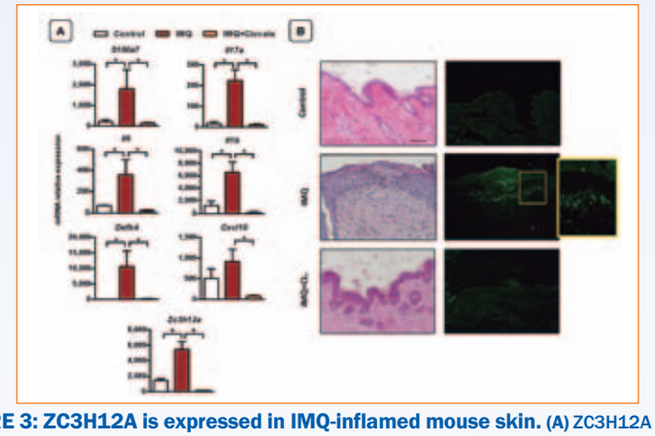

FIGURE 3: ZC3H12A is expressed in IMQ-inflamed mouse skin. **(A)** ZC3H12A mRNA is highly expressed in IMQ-induced psoriasis-like skin inflammation model and it is reduced after topical corticosteroids application (Clovate® cream), as well as other psoriatic genes. **(B)** Immunohistochemistry against ZC3H12A showed specific staining in some abscess-like structures in IMQ-inflamed skin. Scale bar = 50 μm. Yellow square indicates a zoomed region.

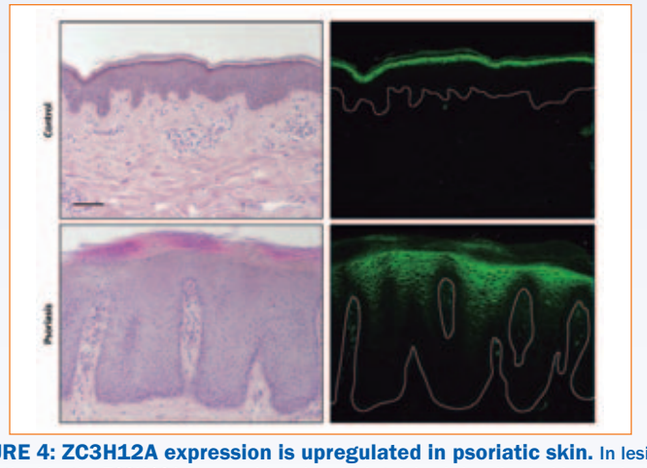

FIGURE 4: ZC3H12A expression is upregulated in psoriatic skin. In lesional psoriatic skin, the ZC3H12A immunohistochemical staining is localized widely and at high levels throughout suprabasal keratinocytes, especially beneath the apical side of the cells, while in control healthy skin is limited to the upper granular layer, thus ZC3H12A expression pattern for the distinct epidermal layers appears to be different. Scale bar= 100 μm.

Conclusion

Our study suggests that ZC3H12A, and its ribonuclease activity, could have an important role in psoriasis disease as it is induced in keratinocytes by supernatants from a psoriatic *ex vivo* model of cocultures using a clinically relevant trigger such as *Streptococcus pyogenes*, mainly through IL-17A. Furthermore the staining pattern in human lesional skin reveals that ZC3H12A shows an expression pattern associated to the abnormal keratinocyte differentiation present in psoriasis.

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