

Expression of COUP-TF-interacting protein 2 (CTIP2) in human atopic dermatitis and allergic contact dermatitis skin

Gitali Ganguli-Indra¹, Xiaobo Liang¹, Stephen Hyter¹, Mark Leid^{1,3}, Jon Hanifin² and Arup K. Indra^{1,3}

¹Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis, OR, USA;

²Department of Dermatology, Oregon Health & Science University, Center for Health and Healing, Portland, OR, USA;

³Environmental Health Sciences Center, Oregon State University, Corvallis, OR, USA

Correspondence: Gitali Ganguli-Indra, Arup K. Indra, Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, USA, Tel: 541-737-9416; 541-737-5775 Fax: 541-737-3999; e-mail: indrag@onid.orst.edu; arup.indra@oregonstate.edu

Accepted for publication 19 February 2009

Abstract: Chicken ovalbumin upstream promoter-transcription factor-interacting protein 2 (CTIP2) is a transcriptional regulator that is highly expressed in skin during mouse development, as well as in proliferating cells of adult mouse skin. We investigated expression of CTIP2 along with proliferation marker Ki-67 in normal human skin, and in skin from atopic dermatitis (AD), and in allergic contact dermatitis (ACD) samples by immunohistochemistry (IHC). We discovered for the first time that CTIP2 was expressed in proliferating basal and suprabasal

layer in normal human epidermis. CTIP2 expression was dramatically increased in the epidermis from the AD and ACD samples compared with normal samples, and was labelled in both proliferating basal and suprabasal layers. Altogether our results suggest that CTIP2 expression could be linked to disease progression and/or maintenance in AD and ACD patients.

Key words: ACD – atopic dermatitis – epidermis – IHC – proliferation – transcriptional regulator

Please cite this paper as: Expression of COUP-TF-interacting protein 2 (CTIP2) in human atopic dermatitis and allergic contact dermatitis skin. *Experimental Dermatology* 2009.

Background

Atopic dermatitis (AD) is an eczematous and highly pruritic, chronic inflammatory skin disease (1–5). A recent, population-based survey in the US recorded a prevalence of 10.7% for non-specific eczema disease and 6% for AD (6). Allergic contact dermatitis (ACD) is caused by cell-mediated hypersensitivity to chemical haptens and is relatively well understood. Although considerable research effort has been directed at AD in the past, this complex disease has challenged a clear understanding of pathogenesis (1,7). A number of factors, such as interactions between susceptibility genes, host environment, altered epidermal proliferation and/or differentiation, skin barrier defects, susceptibility to infection and immunological factors, contribute to the aetiology of AD (1,5,8–12).

Chicken ovalbumin upstream promoter-transcription factor (COUP-TF)-interacting protein 2 (CTIP2), also known as Bcl11b, is a C₂H₂ zinc finger protein that has been shown to regulate transcription through interaction with COUP-TF nuclear receptor proteins as well as through direct, sequence-specific DNA binding (13,14). p57KIP2, a gene encoding a cyclin-dependent kinase inhibitor, has

been identified as a direct transcriptional target of CTIP2 in neuroblastoma cells (15). CTIP2 is expressed early during mouse development as well as in adult animals, and in both cases, expression is most predominant in the skin/epithelial structures, as well as in the CNS and thymus (16,17). CTIP2 expression was detected in the outermost ectodermal cell layer of a developing mouse foetus as early as embryonic day 10.5 (E10.5). In adult mouse skin, CTIP2 expression was found in cells of the basal layer, hair follicles, and in some cells of the suprabasal layers (16). In CTIP2-null mice, the epidermal proliferation/differentiation program was altered and epidermal permeability barrier formation was impaired, leading to thinning of the epidermis (18). Thus, CTIP2 appears to be a critical regulator of skin development in mice. To the best of our knowledge, there has been no study that describes the expression patterns of this important protein in human skin.

Question addressed

What is the expression pattern of CTIP2 in normal human epidermis (NHE) and in skin of hyperproliferative diseases, such as AD and ACD?

Experimental design

Patient samples

Paraffin sections of skin biopsies from 12 AD and 4 ACD samples (all from different patients) were obtained from Oregon Health & Science University, Department of Dermatology, Portland, Oregon. The biopsy specimens were from banked tissue, all taken from lesions of clearly diagnosed, AD adult patients (all of whom also had clinical allergic respiratory disease) or from 48 hrs., contact allergy test sites. IgE was not routinely used for diagnosis of AD, and hence the levels were not available in these patients. The ACD reactions were generated by topical treatment with 2, 4, dinitrochlorobenzene. Matched-skin biopsies from five healthy adults were used as controls. All sample acquisition protocols were approved by the Institutional Review Board at OHSU.

Immunohistochemistry (IHC)

Formalin-fixed and paraffin-embedded sections were used. IHC was performed as described elsewhere (16,19) [see Appendix for detailed protocol].

Results

To characterize CTIP2 expression patterns, we performed IHC staining on histological sections of normal, healthy human skin and on skin biopsies of AD and ACD patients. We observed nuclear staining for CTIP2 mainly in the cells of basal layer and some cells of suprabasal layer of the NHE (Fig. 1a). CTIP2 was expressed in both basal and

suprabasal layers of AD and ACD skin in three independent IHC experiments. No staining was observed in sections stained without primary antibody or with mouse IgG alone (data not shown). Interestingly, CTIP2 expression was dramatically increased in the hyperplastic epidermis of eczematous samples (Fig. 1a). Expression was also detected in infiltrating lymphocytes in the dermis as CTIP2 is known to be expressed by T cells as well (Fig. 1a, AD and ACD, shown by arrowheads). Germline disruption of CTIP2 results in an arrest of T cell development at an immature CD4⁺CD8⁺ stage(s), with the complete absence of $\alpha\beta$ T cells (20). CTIP2-positive cells were found to comprise only $29 \pm 1\%$ of normal, healthy epidermis (Fig. 1b). In contrast, in both AD and ACD samples, numerous CTIP2-positive cells were found throughout the epidermis and their numbers were 60 ± 2 and $52 \pm 1\%$ respectively, of the total epidermal cell population. Thus, our results show that CTIP2 is expressed in basal layer of the NHE, extending all the way up to the suprabasal layer of hyperplastic epidermis from AD and ACD. The number of CTIP2⁺ cells in AD and ACD was significantly higher as compared with normal epidermis ($P < 0.05$), and this correlated well with the hyperplastic nature of diseased skin.

Hyperproliferative skin generally exhibits a high level of expression of the proliferation marker Ki-67 (21–23). To evaluate the proliferation status of CTIP2-expressing cells, we performed co-labelling studies using our anti-CTIP2 monoclonal antibody and an antibody against the proliferation marker Ki-67. All Ki-67 positive cells co-labelled with CTIP2, confirming that all proliferative cells expressed CTIP2 (Fig. 2). A significantly higher number of double-positive cells were present in both basal and suprabasal cell layers of the AD ($26 \pm 1\%$) and ACD ($23 \pm 1\%$) samples compared to normal ($9 \pm 1\%$) skin (Fig. 2b; $P < 0.05$). Altogether, our results suggest that elevated CTIP2 expression might be linked to increased epidermal proliferation in the skin of AD and/or ACD patients.

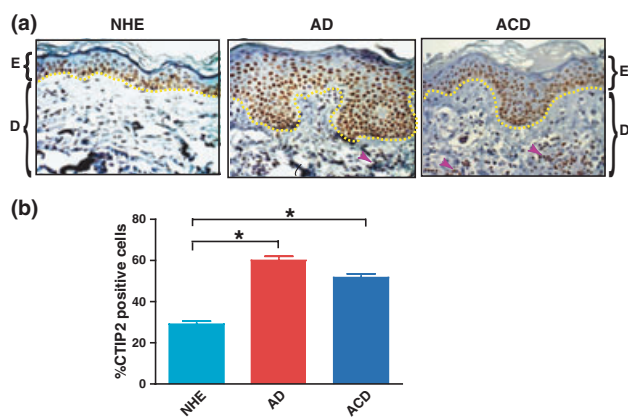


Figure 1. Chicken ovalbumin upstream promoter-transcription factor-interacting protein 2 (CTIP2) expression in human AD and ACD. (a) Analyses of CTIP2 expression by IHC in NHE, AD and ACD skin samples. Brown staining indicates CTIP2-positive cells. Arrowheads indicate dermal CTIP2-positive cells (magnification, 40×). The yellow dotted line defines the epidermal-dermal boundary. E, Epidermis; D, Dermis. (b) The mean frequency of CTIP2-positive cells in NHE, AD and ACD is expressed as percentages. Plotted are mean measurements ($\pm$ SEM). * $P < 0.05$.

Conclusions

In this study, we have demonstrated by IHC that CTIP2 is expressed in NHE. Enhanced expression of CTIP2 was observed in hyperproliferative skin of AD and ACD patients. Co-labelling experiments confirmed CTIP2 expression in all proliferating cells, as well as in some suprabasal cells of the normal and diseased skin. In summary, our results suggest that CTIP2 may play a role in initiation and/or maintenance of proliferative/differentiation state, and possibly in the barrier defects seen in AD, by regulation of genes such as *Id2*, *GATA-3*, *Dgat2* and *eLox-3* (18).

Barrier dysfunction and impaired keratinocyte proliferation and differentiation are crucially involved in the pathogenesis of AD (10,11). Our studies in a mouse model have

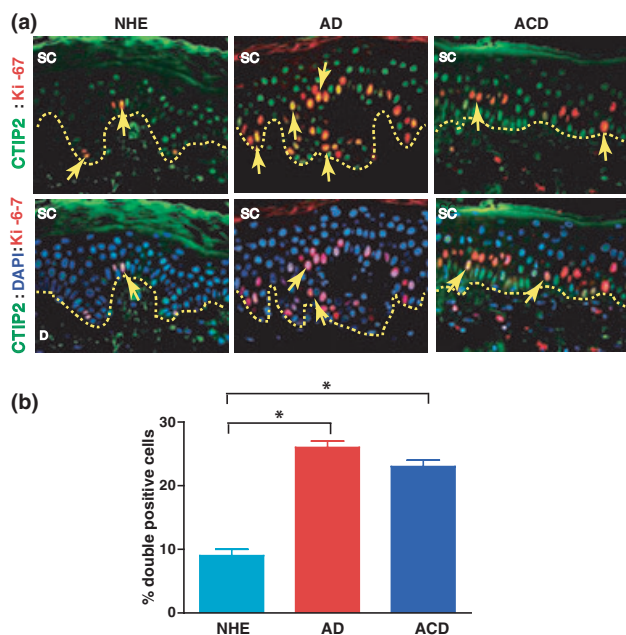


Figure 2. Ki-67 expression in human AD and ACD. (a) Co-labelling of CTIP2 (green), Ki-67 (red) and DAPI (blue) in NHE, AD and ACD samples (original magnification, 40 $\times$). Arrows indicate double-positive cells. SC, stratum corneum (b) The mean frequency of Ki-67⁺CTIP2⁺ double-positive cells in NHE, AD and ACD is expressed as percentages. The mean measurements ($\pm$ SEM) are shown. * P < 0.05.

confirmed that CTIP2 is crucial for the skin barrier formation and for terminal differentiation of keratinocytes during development (18). It is likely that an optimum level of CTIP2 expression is required for the maintenance of skin homeostasis. An increase in CTIP2 expression might lead to an increase in epidermal proliferation and subsequent altered differentiation in AD and/or ACD skin. Additional studies are required to better understand the role of CTIP2 in AD pathogenesis.

Acknowledgements

We thank Adam Campbell and Sophie Truck for help in cell counting during early phase of the project. These studies were supported by Award Number R01AR056008 from the National Institute of Arthritis And Musculoskeletal And Skin Diseases to AI, OSU startup fund (AI) and a NIEHS Center grant (ES00210) to OSU Environmental Health Sciences Center. We also thank Dr Wayne Kradjan and Dr Gary DeLander of the OSU College of Pharmacy for continuous support and encouragement. [Correction added after online publication 29 March 2009: NIH grant number added.]

References

- Bieber T. Atopic dermatitis. *N Engl J Med* 2008; **358**: 1483–1494.
- Leung D Y, Bieber T. Atopic dermatitis. *Lancet* 2003; **361**: 151–160.
- Leung D Y, Boguniewicz M, Howell M D, Nomura I, Hamid Q A. New insights into atopic dermatitis. *J Clin Invest* 2004; **113**: 651–657.
- Simpson E L, Hanifin J M. Atopic dermatitis. *J Am Acad Dermatol* 2005; **53**: 115–128.
- Simpson E L, Hanifin J M. Atopic dermatitis. *Med Clin North Am* 2006; **90**: 149–167.
- Hanifin J M, Reed M L. A population-based survey of eczema prevalence in the United States. *Dermatitis* 2007; **18**: 82–91.
- Simpson E L, Thompson M M, Hanifin J M. Prevalence and morphology of hand eczema in patients with atopic dermatitis. *Dermatitis* 2006; **17**: 123–127.
- De Benedetto A, Agnihothri R, McGirt L Y, Bankova L G, Beck L A. Atopic dermatitis: a disease caused by innate immune defects? *J Invest Dermatol* 2009; **129**: 14–30.
- Ogg G. Role of T cells in the pathogenesis of atopic dermatitis. *Clin Exp Allergy* 2008; **39**: 310–316.
- Proksch E, Brandner J M, Jensen J M. The skin: an indispensable barrier. *Exp Dermatol* 2008; **17**: 1063–1072.
- Proksch E, Folster-Holst R, Jensen J M. Skin barrier function, epidermal proliferation and differentiation in eczema. *J Dermatol Sci* 2006; **43**: 159–169.
- Proksch E, Jensen J M, Elias P M. Skin lipids and epidermal differentiation in atopic dermatitis. *Clin Dermatol* 2003; **21**: 134–144.
- Avram D, Fields A, Pretty On Top K, Nevriy D J, Ishmael J E, Leid M. Isolation of a novel family of C(2)H(2) zinc finger proteins implicated in transcriptional repression mediated by chicken ovalbumin upstream promoter transcription factor (COUP-TF) orphan nuclear receptors. *J Biol Chem* 2000; **275**: 10315–10322.
- Avram D, Fields A, Senawong T, Topark-Ngarm A, Leid M. COUP-TF (chicken ovalbumin upstream promoter transcription factor)-interacting protein 1 (CTIP1) is a sequence-specific DNA binding protein. *Biochem J* 2002; **368**: 555–563.
- Topark-Ngarm A, Golonzhka O, Peterson V J *et al*. CTIP2 associates with the NuRD complex on the promoter of p57KIP2, a newly identified CTIP2 target gene. *J Biol Chem* 2006; **281**: 32272–32283.
- Golonzhka O, Leid M, Indra G, Indra A K. Expression of COUP-TF-interacting protein 2 (CTIP2) in mouse skin during development and in adulthood. *Gene Expr Patterns* 2007; **7**: 754–760.
- Leid M, Ishmael J E, Avram D, Shepherd D, Fraulob V, Dolle P. CTIP1 and CTIP2 are differentially expressed during mouse embryogenesis. *Gene Expr Patterns* 2004; **4**: 733–739.
- Golonzhka O, Liang X, Messaddeq N *et al*. Dual role of COUP-TF-interacting protein 2 in epidermal homeostasis and permeability barrier formation. *J Invest Dermatol* 2008; Dec. 18.
- Ganguli G, Abecassis J, Wasyluk B. MDM2 induces hyperplasia and premalignant lesions when expressed in the basal layer of the epidermis. *EMBO J* 2000; **19**: 5135–5147.
- Wakabayashi Y, Watanabe H, Inoue J *et al*. Bcl11b is required for differentiation and survival of alphabeta T lymphocytes. *Nat Immunol* 2003; **4**: 533–539.
- Kawahira K. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in malignant and nonmalignant skin diseases. *Arch Dermatol Res* 1999; **291**: 413–418.
- Sapuntova S G, Mel'nikova N P, Deigin V I, Kozulin E A, Timoshin S S. Proliferative processes in the epidermis of patients with atopic dermatitis treated with thymopressin. *Bull Exp Biol Med* 2002; **133**: 488–490.
- Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol* 2000; **182**: 311–322.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Immunohistochemistry (IHC).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Supporting materials

Appendix S1

Immunohistochemistry (IHC)

Paraffin sections (5 μm) were deparaffinized in xylene, rehydrated through graded alcohols, and placed in 0.1% hydrogen peroxide to quench any endogenous peroxidase activity. Sections were subjected to four rounds of an antigen retrieval technique (5 min in a 750 W microwave pretreatment in citrate buffer, pH 6.0), followed by treatment with 10% normal goat serum for 30 min. to block nonspecific antibody binding. The slides were then incubated with rat CTIP2 monoclonal antibody, 1/300 dilution (Abcam product number 18465; clone 25B6) in a humid chamber at 4°C overnight. Skin sections from CTIP2-null mice (since the CTIP2 antibody reacts with mouse CTIP2) (18), and sections incubated with IgG alone (isotype control) or without primary-antibody, were used as controls. The secondary staining was carried out using a biotin-labeled, rabbit anti-rat antibody at 1/500 dilution (Jackson Immuno Research Laboratories, Inc., catalogue number 112-065-143) for 2 hrs. at 37°C, followed by incubation with a streptavidin-conjugated horseradish peroxidase (Vector Laboratories, catalog number: SA-5704). We observed the reaction products by immersing the slides in freshly prepared diaminobenzidine solution for 10 min. and counterstaining with hematoxylin before dehydration and mounting. Images were captured at 40X magnification using Leica microscope and Hamamatsu C4742-95 digital camera, and processed using OpenLab software and Adobe Photoshop 7.0. We counted

the number of CTIP2-positive cells as a percentage of H&E positive cells (total cells) within a field of fixed size.

For immunofluorescence studies, paraffin sections were processed as described above. Slides were then incubated overnight in a humidified chamber with anti-CTIP2 and -Ki-67 (Abcam product number ab15580; 1:200 dilution) antibodies. Skin sections from CTIP2-null mice, and sections incubated without primary-antibody, or IgG alone (isotype cptrls) were used as controls. The primary antibody incubation was followed by three washes with PBST and incubation with fluorescently-labeled (Cy2-donkey anti-rat [1:250] for CTIP2 and Cy3-goat anti-rabbit for Ki67 [1:500]; Jackson Immuno-Research] secondary antibodies for 2 h. Nuclei were counterstained with DAPI. Finally, sections were rinsed with PBST, dehydrated through sequential washes in 50%, 70%, 95%, and 100% ethanol and then cleared in xylene. Slides were mounted with DPX mounting media and allowed to dry overnight. Images were captured at 40X magnification using Leica DMRA fluorescent microscope and Hamamatsu C4742-95 digital camera and processed as above. CTIP2⁺Ki67⁺ double positive cells within a field of fixed size were quantified and expressed as a percentage of DAPI+ cells (total cells) in that same field. The expression analyses and cell counts were independently performed by two investigators.

Statistical analysis

Cell counts were performed on ten different fields at a fixed magnification for each sample (5 normal, 12 AD, and 4 ACD samples). Data are presented as

mean $\pm$ SEM. Data were analyzed by unpaired, 2-tailed t test and p values of < 0.5 were considered significant.