



## CORRESPONDENCE

### Psoriasis is characterized by altered epidermal expression of caspase 14, a novel regulator of keratinocyte terminal differentiation and barrier formation

#### KEYWORDS

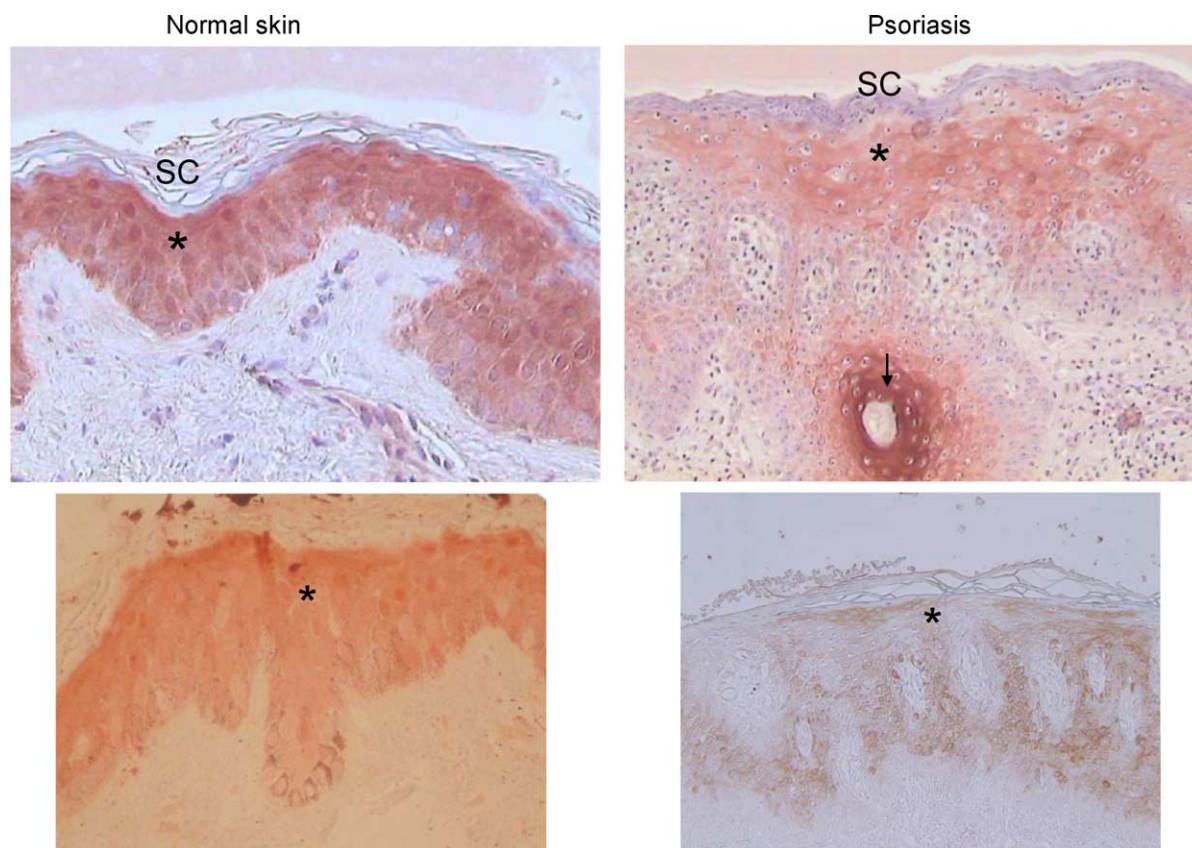
Caspase;  
 Caspase 14;  
 Psoriasis;  
 Epidermis;  
 Keratinocytes

Caspases are a family of cysteine proteases involved in the effector arm of physiologic cell death [1]. In 1998, a novel caspase designated “caspase 14” was described in embryonic and adult tissues, especially epidermal keratinocytes [2–4]. Unlike other caspases (such as 3, 6 and 7), caspase 14 is not processed by typical death stimuli or activated during apoptosis induced by ultraviolet irradiation or cytotoxic substances. However, caspase 14 is cleaved under conditions leading to terminal differentiation, suggesting a unique role in keratinocyte “planned cell death” in forming the stratum corneum [2,5]. We postulated that psoriasis, a chronic papulosquamous disease with aberrant epidermal proliferation and terminal differentiation [6], may express altered levels of caspase 14.

Here, we used immunohistology to compare caspase 14 expression in paraffin embedded sections of normal and psoriatic skin samples. Skin biopsies were obtained under an approved human use protocol after signed informed consent. Skin samples were fixed in 10% neutral buffered formalin and then paraffin embedded. Normal ( $n = 3$ ) and psoriatic ( $n = 6$ ) skin samples from different patients were analyzed for caspase 14 presence using a modified avidin-biotin-peroxidase immunohistochemical technique [7]. The 5  $\mu\text{M}$  paraffin sections placed onto positively charged glass slides were deparaffinized in limonene (Sigma, St. Louis, Missouri, USA) and rehydrated in a descending series of ethanols to water. Endogenous peroxidase activity was blocked by a 5-min incubation in 0.3%  $\text{H}_2\text{O}_2$ . Non-specific binding of the antibody to tissue sections was

blocked by incubation for 1 h in 10 mg/mL bovine serum albumin. Rabbit anti-caspase 14 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) diluted 1:50 was applied to the sections for 1 h. Control sections were processed identically except that normal rabbit serum was substituted for anti-caspase 14 antibody. After washing in PBS, a 1:200 dilution of the secondary biotin-conjugated goat anti-rabbit immunoglobulin antibody (Vector Laboratories, Burlingame, California, USA) was applied for 30 min. After further washing in PBS, the tissue sections were incubated for 30 min in an avidin-peroxidase complex reagent (ABC reagent, Vector, Burlingame, California, USA). Following three PBS washes, Nova-Red substrate (Vector, Burlingame, CA) was added to produce a red reaction product over sites of antibody binding to caspase 14. Most sections were counterstained with Mayer’s hematoxylin as an aid to morphological identification, whereas some experiments omitted the counterstain to better observe cellular localization of caspase 14. After staining, tissue sections were dehydrated in ascending concentrations of ethanol to xylene and coverslipped with Cytoseal XYL (Stephens Scientific, Riverdale, New Jersey, USA). All stained sections were viewed with light microscopy, assessed, and photographed.

All normal skin sections showed caspase 14 staining in the epidermis, with mild staining in the basal region and more prominent staining in the suprabasilar cells involving the cytoplasm and nuclei (Fig. 1, left, upper). All psoriatic sections showed reduced or patchy caspase 14 throughout the epider-



**Fig. 1** Caspase 14 epidermal staining, denoted by red color (\*), in normal (left, upper) and psoriatic (right, upper) skin samples, counterstained with hematoxylin ( $\times 200$ ). Normal skin shows intense supra-basilar caspase 14 staining involving cytoplasm and nuclei, and a well-formed, "basket-weave" stratum corneum (SC). The psoriasis section shows reduced, patchy epidermal caspase 14 staining, with prominent, unstained nuclei, and poorly formed stratum corneum (SC). Arrow denotes caspase 14 in a hair follicle, with staining at least as intense as normal supra-basilar epidermis, especially adjacent to the keratinizing hair shaft. Lower images (left and right) show normal and psoriatic samples, respectively, stained for caspase 14, without hematoxylin counterstain (\*). Normal skin shows caspase 14 in cytoplasmic and nuclear regions, whereas psoriatic epidermis shows patchy and notably reduced staining, especially in nuclear regions.

mis, especially over parakeratotic areas with poorly formed stratum corneum (Fig. 1, right, upper). In many cells, there were many prominent nuclei without caspase 14 stain. A hair follicle in one psoriatic sample showed caspase 14 staining at least as intense as the supra-basilar epidermal cells of normal skin, with the darkest staining adjacent to the keratinizing hair shaft (Fig. 1, right, upper). In selected samples without hematoxylin counterstain, normal epidermal cells showed caspase 14 expression in a near-confluent pattern in most supra-basilar cells involving cytoplasmic and nuclear regions (Fig. 1, left, lower). The supra-basilar cells of the psoriatic samples showed patchy staining and prominent unstained nuclei (Fig. 1, right, lower).

In accordance with earlier work, supra-basilar epidermal cells in normal skin samples expressing caspase 14 are consistent with the notion caspase 14 may play a role in regulating terminal differentiation of keratinocytes [8]. In comparison, sections of psoriasis, a skin disorder characterized by increased

epidermal cell proliferation with abnormal terminal differentiation, showed altered caspase 14 expression, with decreased or patchy cytoplasmic staining and prominent, unstained nuclei. Interestingly, an epithelium-covered hair follicle in a psoriatic sample showed a staining pattern similar to normal epidermis, whereby terminally differentiating cells closest to the hair shaft showed more intense caspase 14 staining. This observation suggested that caspase 14 deficiency in psoriasis may be restricted to the epidermal cells.

Altered caspase 14 expression in psoriasis, with apparent involvement of both the cytoplasm and nuclei, is a new biochemical aberration of psoriasis that may have implications in planning alternate strategies for therapy. For example, Hsu et al. reported that green tea polyphenols induce accelerated terminal differentiation of cultured human epidermal keratinocytes [9], in association with markedly upregulated caspase 14 expression (S. Hsu, personal communication).

Our findings are consistent with those of Lippens et al. who showed that caspase 14 expression in psoriasis was markedly reduced [5], especially in areas of parakeratosis, a histological marker of abnormally high epidermal proliferation resulting in faulty barrier formation. In addition, we noted that caspase 14 staining in normal epidermal cells appeared to involve the cytoplasm and nuclei, whereas in psoriasis, nuclear staining was clearly reduced or even absent in some areas. This suggests that, in addition to cytoplasmic caspase 14, nuclear translocation of caspase 14 may be relevant to normal barrier formation. With recent evidence that nucleocytoplasmic transport of related caspase 3 is necessary for dismantling nuclear pores during apoptosis [10], a key step in apoptosis, we postulate that caspase 14 may also play an important role as it enters the nuclei of keratinocytes committed to terminal differentiation. In psoriasis, reduced nuclear entry of caspase 14 may lead to faulty terminal differentiation.

Caspase 14 has so far only been isolated from the cytosol. However, in light of our observations and the relationship between nucleocytoplasmic transport of caspase 3 and apoptosis [10], we speculate that psoriatic keratinocytes may activate mechanisms that prevent the nuclear entry of caspase 14, thereby inhibiting physiologic barrier formation. Future work will focus on understanding the implications of deficient caspase 14 in psoriasis, including the use of animal models to manipulate caspase 14 for further biochemical and clinical insight.

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Douglas S. Walsh\*

*Dermatology Service, 300 E Hospital Road  
Ft. Gordon, GA 30905, USA*

*Department of Clinical Investigation  
Dwight D. Eisenhower Army Medical Center  
Fort Gordon, GA, USA*

James L. Borke

Baldev B. Singh

Nah-Nam Do

Stephen D. Hsu

*Department of Oral Biology and Maxillofacial  
Pathology, School of Dentistry  
Medical College of Georgia, Augusta, GA, USA*

Maria V. Balagon

Rodolfo M. Abalos

*Leonard Wood Memorial Center for Leprosy  
Research, Cebu City, Philippines*

\*Corresponding author. Tel.: +1 706 787 1472

fax: +1 706 787 1354

*E-mail address: douglas.walsh@se.amedd.army.mil  
(D.S. Walsh)*

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