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**Letter to the Editor, Journal of Dermatological Science**

**Possible role of endoplasmic reticulum stress in the pathogenesis of Darier's disease.**

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Darier's disease (DD, keratosis follicularis; OMIM 124200) is an autosomal dominant genodermatosis characterized by persistent, greasy, scaly papules which show abnormalities in keratinocyte adhesion and differentiation including acantholysis, suprabasal clefting, and unusual dyskeratosis. Mutations within the *ATP2A2* gene encoding the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase type 2 (SERCA2) are found in DD patients, indicating that SERCA2 plays an important role in keratinocyte adhesion and differentiation [1]. However, the precise mechanisms underlying the histological hallmarks in DD have not yet been fully elucidated.

The endoplasmic reticulum (ER) may serve specialized functions including the post-translational modification, folding and assembly of newly-synthesized secretory proteins. Various conditions can interfere or disrupt ER function, and these are collectively grouped into ER stress-associated diseases. ER stress provokes an ER stress response, which includes upregulation of ER chaperones, inhibition of gene translation, degradation of the misfolding proteins and induction of a transcription factor C/EBP homology protein (CHOP) that leads to cell apoptosis [2].

SERCA2 actively transports  $\text{Ca}^{2+}$  from the cytosol back into the ER lumen to maintain the correct  $\text{Ca}^{2+}$  concentration in the ER. ER stress can be induced by a decrease in  $\text{Ca}^{2+}$  concentration within the ER. These suggest that SERCA2 abnormalities in DD are caused by the low  $\text{Ca}^{2+}$  in the ER lumen, resulting in ER stress. This study was designed to address a hypothesis that ER stress is involved in formation of the characteristic histological features of DD.

The lesional skin specimens and cultured keratinocytes obtained from a 17-year-old female with DD were used for study. Diagnosis of DD was determined by dermatologists based upon clinical and histopathological features (The mutation C318R in *ATP2A2* was previous reported [3]). The normal human keratinocytes and skin specimens were obtained from a normal adult female. In order to observe the ER stress response in keratinocytes, we examined the expression of calreticulin [4], BiP/GRP78 [5] and CHOP/gadd153 [6]. The primary keratinocyte cultures were grown in serum-free keratinocyte growth medium (KGM, Clonetics) and then treated with the stressors, 1.0  $\mu\text{M}$  thapsigargin [7] or 1.0 mM S-nitro-N-acetyl-DL-penicillamine (SNAP) [8] for 48 hours.

The specimens were embedded in OCT compound, and 10  $\mu\text{m}$  thick

sections were cut. The treated keratinocytes and cryosections were stained with rabbit polyclonal antibodies against calreticulin (Stressgen), rabbit polyclonal antibodies against or CHOP/GADD153 (Santa Cruz) or goat polyclonal antibodies against BiP/GRP78 (Stressgen), followed by treatment with FITC-conjugated secondary antibodies. To semiquantify the keratinocytes expression of calreticulin, CHOP/GADD153 and BiP/GRP78 respectively, we measured fluorescence intensity of each cell using the digital photograph (Gel Plotting Macros; NIH Image ; provided in the public domain by the National Institutes of Health, Bethesda, MD, and available at <http://rsb.info.nih.gov/nih-image/>). We graded the fluorescence intensity of stained cells as follows; Level 1: poor staining, Level 2: moderate fluorescence levels, Level 3: bright cytoplasmic fluorescence. Typical staining pattern of each Level was shown in Fig. 1A. Intensity value was estimated from;  $(\text{the number of level 1 cells} \times 1) + (\text{the number of level 2 cells} \times 2) + (\text{the number of level 3 cells} \times 3) / \text{the total number of the cells}$ , and repeated the same experiment four times. Finally, the expression ratio was represented as the ratio of intensity values of stimulated to unstimulated samples.

The results of the culture cell study are shown in Fig. 1B. The expression of three molecules in DD keratinocytes was all higher than that in normal keratinocytes. Significant differences ( $p < 0.02$ ) were found in thapsigargin-induced calreticulin, CHOP/GADD153 and BiP/GRP78 samples and SNAP-induced calreticulin sample.

Next, we examined the expression of calreticulin, CHOP/GADD153 and BiP/GRP78 in DD lesional skin. Hematoxylin and eosin sections clearly showed the dyskeratotic cells and corps ronds with the suprabasal cell layers. These cells stained with anti-calreticulin antibody (Fig 2). We however failed to find immunostaining in any of the Anti- Bip/GRP78 and CHOP/GADD153 in sections (data not shown). Skin section from normal control showed no immunoreactivities for any of those molecules.

The ER stress response is a mechanism by which cells protect themselves against ER stress. One response involves the up-regulation of genes encoding ER chaperone proteins to increase protein folding activity and to prevent protein aggregation. When the functions of the ER are severely impaired, apoptosis is induced via the transcriptional induction of CHOP/gadd153. The ER stress inducers, thapsigargin and SNAP were added to the keratinocyte cultures because both the DD and normal keratinocytes without any stressors

showed a relatively low level of expression of above three ER chaperons. Those stressors induced ER stress by different mechanisms. This study showed that the levels of ER chaperons calreticulin, CHOP/gadd153 and Bip/GRP78 were increased in DD keratinocytes compared with normal control keratinocytes, suggesting that ER stress might be somehow involved with pathogenesis of Darier's disease.

Hakuno reported that the dissociation of intra- and extracellular domains of desmosomal cadherin and E-cadherin are characteristics of acantholytic cells in DD [9], this phenomenon might be explained by ER stress leading to important protein misfolding or misassembly. In addition, we found strong expression of calreticulin in dyskeratotic cells in DD lesional skins. Although we observed little or no detectable expression of CHOP/gadd153 in these cells, such dyskeratotic cells might result from apoptosis induced by ER stress.

This study suggests that the ER stress may be involved in the pathogenesis of DD. The treatment of DD has mainly included oral retinoids, or topical retinoids for localized DD. Recently some agents are shown to have an inhibitory effect on ER stress in the other organs [10], so we suggest that drugs which control ER stress in keratinocytes might hold significant potential for the treatment of DD.

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### **Legends for Figures**

**Figure 1** Expression of calreticulin, CHOP/GADD153 and BiP/GRP78. DD (■) and normal (□) keratinocytes were treated with thapsigargin and SNAP for 48 hours, and expression of calreticulin (Cal) CHOP/GADD153 (CHOP) and BiP/GRP78 (Bip) was scored and quantified as intensity value. A) We counted to a hundred of the cells in a chamber, and classified as follows; Class 1: dark and poor staining in the cytoplasm (a). Class 2: moderate green deposit in the cytoplasm (b). Class 3: very bright cytoplasm with yellow colored (c). B) DD keratinocytes induced expression of calreticulin, CHOP/GADD153 and BiP/GRP78 more than normal keratinocytes. Expression ratio was represented as the ratio of intensity values of stimulated to unstimulated cell samples. The results were plotted as a mean  $\pm$  SD. \* : Significant differences between DD and normal samples ( $p < 0.02$ ).

**Figure 2:** Immunohistochemical analysis of DD skin. (a): dyskeratotic cells, corps ronds (arrows), suprabasal clefts (\*) and acantholytic keratinocytes were observed. (H&E staining). (b): Expression of calreticulin was detected in dyskeratotic cells (arrows). Blue arrowheads indicate the position of the basement membrane.

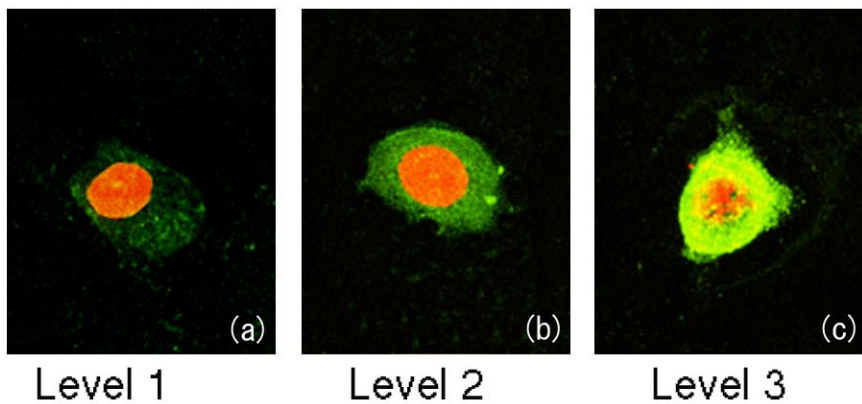
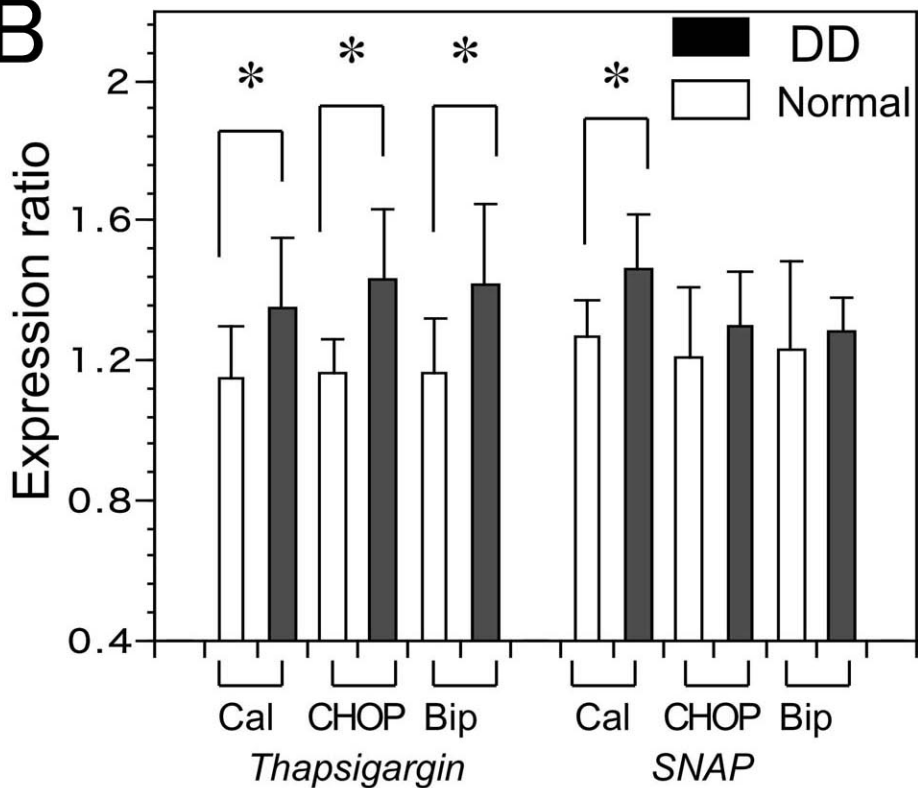
**A****B**

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