

SHORT COMMUNICATION

Heat Shock Protein 27 kDa Content in Stratum Corneum Correlates with Severity of Atopic Dermatitis

Shiro Niiyama¹, Takashi Yoshino², Shoko Matsukuma² and Hideki Mukai¹¹Department of Dermatology, Toho University Ohashi Medical Center, 2-17-6 Ohashi, Meguro-ku, Tokyo 153-8515, Japan and ²Fanci Research Institute, Yokohama, Kanagawa, Japan. E-mail: sniiyama@aol.com

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Several proteins in tape-stripped samples of the stratum corneum (SC) have been reported recently as biomarkers for various skin conditions. Thymus- and activation-regulated chemokine (1), vascular endothelial growth factor (2), interleukin (IL)-8 (3), fatty acid-binding protein-5 (4), nerve growth factor (5), thymic stromal lymphopoietin (6) and macrophage migration inhibitory factor (7) have been reported as biomarkers in SC that reflect the skin signs/symptoms of patients with atopic dermatitis (AD).

Heat shock protein 27kDa (HSP27) belongs to the phylogenetically conserved small heat shock protein family, which can act as molecular chaperones and protect cells from heat shock and oxidative stress *in vitro*. HSP27 is constitutively expressed in the normal skin and confined mainly to the upper epidermal layer (8). Ultraviolet-B irradiation, heating, irritation with methylparaben and sodium lauryl sulphate induced the expression of HSP27 in human keratinocytes or excised skin (9). These reports suggest the existence of a correlation between environmental stress and HSP27 expression in the skin, and it is speculated that HSP27 may be one of the factors promoting repair responses in inflammation occurring in response to environmental stress, including inflammatory skin diseases, such as AD. This study used tape-stripping to make serial measurements of the HSP27 content of SC in AD patients.

MATERIALS AND METHODS

A total of 34 patients with AD (21 men and 13 women, age range 20–60 years, mean 33.3 years) were enrolled. The local severity scores of the involved skin were calculated based on the scores for xerosis, excoriation, erythema, papules, pruritus, and oozing, each of which was evaluated on a 5-point scale (0: none, 1: very mild, 2: mild, 3: moderate, and 4: severe) on the patient's cheek (10). The value is the median score of all 6 parameters. All patients were treated with topical corticosteroids and oral antihistamines. Corticosteroid treatment was discontinued 12–24 h before the examination day, and the patient washed their whole body in the shower, without soap, for 5–10 min on the day before the tape-stripping. In the 15 inpatients, serial measurements were made: 3 times at 3-day intervals. Samples were taken from the remaining 19 outpatients at only 1 time-point.

SC samples were obtained by single-stripping using a skin tape (25 × 25 mm; Horney Layer Checker; Asahi Biomed Co. Ltd, Tokyo, Japan). Samples were extracted with T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific Inc., Rockford, IL, USA). Protein concentrations in the extracts were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The HSP27 levels in the extracts were measured using

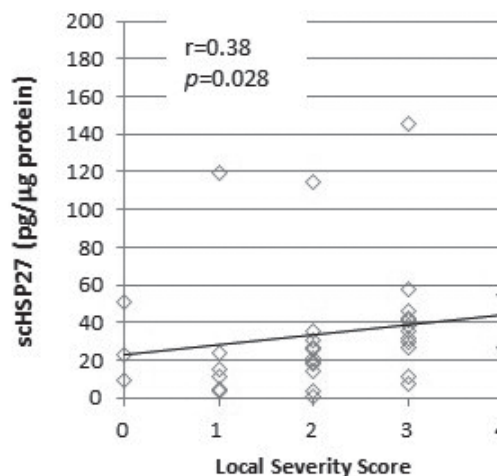


Fig. 1. Correlation of the local severity scores on the cheek with the HSP27 contents in tape-stripping samples from the cheek ($n=34$).

an enzyme-linked immunosorbent assay (ELISA) kit (Human HSP27 DuoSet, R&D Systems, Inc., Minneapolis, MN, USA).

All statistical analysis was performed using the Spearman's rank correlation test and the Kruskal-Wallis test with Bonferroni's correction ($p < 0.05$).

RESULTS

The results revealed the existence of significant correlations between the severity scores of the AD lesions at the start of the study and the HSP27 in SC (scHSP27) contents (Fig. 1). Analysis of the changes in response to inpatient treatment showed that, by the end of the 3 measurements, the severity scores had greatly improved, and that the scHSP27 contents had decreased in parallel with the scores (Fig. 2).

DISCUSSION

This study showed, for the first time, that HSP27 values in patients with AD change in parallel with changes in skin symptoms. Studies that have used the tape-stripping method have only reported comparisons between patients and healthy subjects, or between affected and unaffected sites in patients with AD, whereas in the present study, we demonstrated that the HSP27 values changed in parallel with the changes in skin symptoms in the same patients. Ghoreishi et al. (11) reported that the HSP27 expression levels were

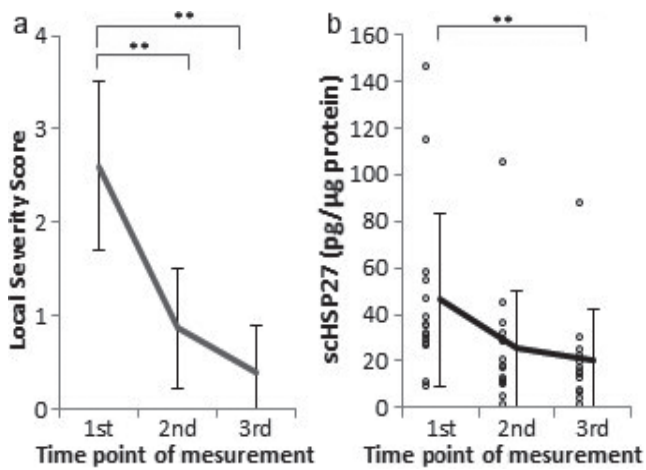


Fig. 2. Changes in (a) local severity scores and (b) scHSP27 contents for lesions on the cheek of inpatients with AD ($n=15$). Stratum corneum (SC) samples were collected at the time of admission (1st), 3 days after (2nd), and at the time of discharge (3rd). Line plots show the mean and error bars show the standard deviation. ** $p<0.01$.

not correlated with the severity of AD. However, they evaluated HSP27 expression levels in the epidermal tissue by immunohistochemical staining, whereas in our study we extracted HSP27 from the SC and assayed it by ELISA, which has a higher detection sensitivity.

Profilaggrin is a ~400 kDa human protein that is critical for normal skin barrier development. It is principally expressed in a differentiation-dependent manner in the stratum granulosum. Loss-of-function mutations in the profilaggrin gene associate it with skin diseases, including AD (12). Furthermore, variation in the copy number of the filaggrin monomers within the profilaggrin gene is associated with a dry skin phenotype in the general population (13). Recently, Bunick et al. (14) demonstrated that HSP27 binds to the N-terminal domain of human profilaggrin, and O'Shaughnessy et al. (15) reported that the loss of HSP27 is associated with hyperkeratinization and misprocessing of filaggrin. Our data showed that the HSP27 expression levels in the skin were elevated in patients with AD. The results strongly suggest that HSP27 expression may become markedly elevated as a biological defence response to environmental stress occurring secondarily to barrier disruption in AD. Because we did not investigate the genotype and phenotype profile of filaggrin in the present study, it could not be determined whether the filaggrin expression level returned to normal in association with increased HSP27 expression. Therefore, a future research task is to analyse the correlations among the scHSP content, and profilaggrin and filaggrin expression levels at inflammatory and non-inflammatory sites in patients with AD. Multiple factors besides the lack of the profilaggrin gene may be involved in the reduced expression of profilaggrin in patients with AD, and HSP27 may be one such factor.

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