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HSF1 is Important for Protection of Mouse Epidermal Cells against Heat Stress

Chie Nakamura,[1)2)] *Yoshitaka Nakamura,*[1)2)] *Toshikazu Gondo,*[3)] *Eiichi Takaki,*[1)] *Mitsuaki Fujimoto,*[1)] *Sachiye Inouye,*[1)] *Akira Nakai*[1)] *and Masahiko Muto*[2)]

[1)] Department of Biochemistry and Molecular Biology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2)] Department of Dermatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[3)] Department of Pathology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
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Abstract Heat shock transcription factor 1 (HSF1) is essential for transcriptional activation of heat shock genes in response to heat shock in mammals, and is also involved in developmental processes. However, little is known about whether HSF1 regulates expression of heat shock proteins (Hsps) in the epidermis in the presence or absence of heat shock. Here we found that Hsps (Hsp110, Hsp90, Hsp70, Hsp60, Hsp40 and Hsp27) are expressed in unstressed epidermal cells of mouse, mostly by HSF1-independent pathway. To clarify the distribution of Hsp expression in mouse epidermis, we have approached this issue using an *in vivo* mouse model with chronic cutaneous inflammation and irritation by epicutaneous painting 5% sodium dodecyl sulfate (SDS). We found that Hsp70 and Hsp27 are highly expressed in the upper epidermal layers, suggesting that their expression is related to keratinocyte differentiation. Hsp70 was highly induced in the mouse epidermis when the skin was exposed to heat shock *in vivo*. HSF1 is required for its induction, and HSF1-null epidermal cells were more sensitive to heat shock than wild-type cells. These results indicate that HSF1 is important for protection of mouse epidermal cells against heat stress.

Key words: epidermis, heat shock protein, HSF, mouse, transcription

Introduction

Exposure of cells to elevated temperatures induces a physiologic response characterized by the synthesis of a specific set of proteins called heat shock proteins (Hsps) that mediate repair and protection from cellular damages.[1)] Expression of Hsps is mainly regulated at the level of transcription by heat shock transcription factors (HSFs).[2)] Among of the HSFs, HSF1 is required for heat shock response and protection from cell death under the primed condition of heat shock,[3-5)] and protection of cells from various pathophysiological conditions such as infarction, acoustic damage, and gastric ulcer.[6-8)] However, little is known about whether HSF1 regulates the expression of Hsps in the epidermis in the presence or absence of heat shock.

The mouse and human epidermis are a surface barrier of the body and are continuously exposed to various environmental stresses. Heat shock response can be a major adoptive response in the skin.[9)10)] Hsps are expressed in unstressed cultured human keratinocytes *in vitro* and epidermal cells *in vivo*, and are induced in response to physical stresses such as high temperatures and ultra-

violet light.[9)11-15)] To understand the role of HSF1 that regulates heat shock gene expression in the skin, we developed HSF1-null mice as tool and analyzed the epidermis of the mice under heat shock-primed condition. Our results certainly show that HSF1 is essential for protection of the epidermal cells through increased expression of Hsps, especially Hsp70 against thermal stress.

Materials and methods

In vivo mouse model of chronic cutaneous irritation

The HSF1-null mice were generated previously,[5)] and were maintained by crossing into ICR mice. Mice were anesthetized by intraperitoneal injection of 25 μg/kg sodium pentobarbital (Dainippon Pharmaceutical Co., Osaka, Japan), and the backs were shaved with an electric trimmer. Then, 5% sodium dodecyl sulfate (SDS) in phosphate-buffered saline (PBS) was painted epicutaneously once per day for 5 days.

Exposure of the mouse skin to heat shock

Those SDS-treated mice were anesthetized by intraperitoneal injection of 25 μg/kg sodium pentobarbital, placed on hot plate at 50 ℃ for 3 min, and then allowed to recover at room temperature. Experimental protocols were reviewed by the Committee for Ethics on Animal Experiments of Yamaguchi University School of Medicine.

Histological examination and immunostaining

The dorsal skin of the mouse was dissected, fixed in 4% paraformaldehyde for several hours, and then embedded in paraffin. Sections of 4 μm thick were stained with hematoxylin and eosin (HE). Percentages of the epidermal region having subepidermal blister were estimated from three independent experiments. Immunostaining of the paraffin sections was performed as described previously.[27)] Antibodies used were rabbit antisera for mouse Hsp110(αHsp110a), human Hsp90(αHsp90c), human Hsp70(αHsp70-1), human Hsp40(αHsp40-1), mouse Hsp27(αmHsp27c),[31)] and mouse Hsp60(αmHsp60-1).[32)] Peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG was used as a second antibody. Signals were detected using a DAB substrate kit (Vector Laboratories, Inc., CA).

Western blot analysis and gel shift assay

HeLa cells were maintained in DMEM medium containing 10% fetal bovine serum at 37 ℃ in 5% CO_2 incubator. Normal human epidermal keratinocytes (NHEK) were maintained at 37℃ in EpiLife medium containing 10 μg/ml insulin, 0.1 ng/ml human epidermal growth factor, 0.5 μg/ml hydrocortisone, and 0.4% bovine pituitary extracts (Kurabo Co., Osaka, Japan). Cells were treated with heat shock at indicated temperatures, and cell extracts were prepared using buffer C containing 20 mM HEPES (pH 7.9), 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 1 μg/ml leupeptin and 1 μg/ml pepstatin A.[27)] Aliquots containing of proteins were subjected to Western bolt analysis using rabbit serum for human HSF1 (αhHSF1e),[33)] Hsp70 (αHsp70-1), and β-actin (AC-15, Sigma). Gel shift assay was performed using using a ^{32}P-labelled HSE-oligonucleotide as a probe.[27)]

Results

Hsps are expressed in unstressed epidermal cells, mostly by HSF1-independent pathway

We examined histology of the back skin of both 6-week-old wild-type and HSF1-null mice. The epidermis consists of one to two cell layers in wild-type skin, and overall structures of the skin were unaffected in HSF1-null skin (Fig. 1A, a, b). We then analyzed protein levels of Hsps by an immunohistochemical staining. As was reported previously in human[15)] or mouse[14)] epidermis, major Hsps, which include Hsp110, Hsp90, Hsp70, Hsp60, Hsp40, and Hsp27, are expressed in most unstressed wild-type epidermal cells (Fig. 1A, c-n). Expression levels of Hsp110 and Hsp40 are a little lower in HSF1-null epidermal cells, but the expression levels did not change significantly. Levels of other Hsps are constant in HSF1-null epidermal cells. These results indicate that Hsps are expressed in unstressed epidermal cells, mostly by HSF1-independent pathway.

In contrast to human epidermis that consists

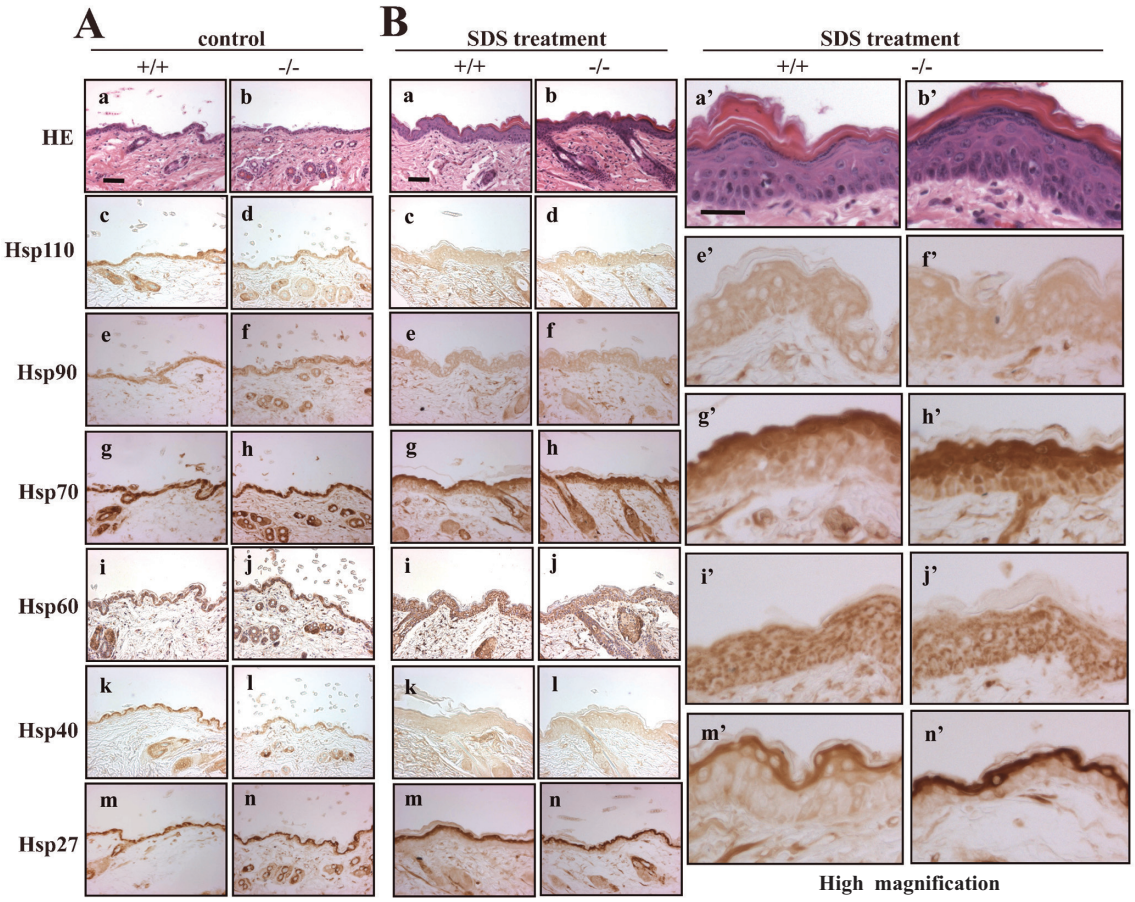

Fig. 1 Immunohistochemical analysis of Hsps in control and SDS -treated skin in wild-type and HSF1-null mice.
Biopsy samples were taken from anesthetized wild-type (+/+) and HSF1-null (-/-) mice prior to (control, A) and following (SDS, B) SDS-treatment in vivo. Skin sections (4μm in thickness) were stained with hematoxylin and eosin (a, b). Immunostaining was performed using monoclonal antibodies specific for mouse Hsp110, Hsp90, Hsp70, Hsp60, Hsp40 and Hsp27 (c-n). Some are shown in high magnification pictures (a'-n').

of several different cell layers, the epidermis in mouse back skin is very thin, as is described above. As a result, it is hard to determine subcellular localization and differentiation-related expression of Hsps and to detect cell death in normal mouse skin. To examine these aspects more precisely, we used an *in vivo* model of chronic cutaneous inflammation and irritation by daily epicutaneous painting with 5% SDS. In this model, disruption of barrier function by SDS induces infiltration of granulocytes and macrophages that promote epidermal hyperproliferation and keratosis. We found hyperproliferation of epidermal cells and keratosis in both wild-type and HSF1-null mice (Fig. 1B, a, b, a', b'). More precisely, acanthosis and thickening of the horny layer was observed in both wild-type and HSF1-null epidermis. Furthermore, nuclei of HSF1-null keratinocytes were more strongly stained by hematoxylin than those of wild-type keratinocytes, and keratohyaline granules in the granular layer of the HSF1-null mice were denser than those of wild-type mice, suggesting a slight abnormal differentiation in the HSF1-null epidermis. However, overall structures of SDS-treated epidermis are similar in wild-type and HSF1-null mice.

Hsp70 and Hsp27 expressions were mainly in the granular cell layer in SDS-treated wild-type and HSF1-null mouse epidermis

We performed an immunohistochemical analysis of major Hsps in wild-type epidermis without heat shock. Among them, Hsp70 and Hsp27 staining was stronger in the granular cell layer than in the epidermal deep layer (Fig. 1B, c-n, e'-n'), indicating that these Hsps are involved in terminal differentiation of keratinocytes.[16-18] In contrast, staining of Hsp110, Hsp90, Hsp60, Hsp40 was observed the same in all cell layers. Most Hsps are stained in the cytoplasm (Hsp90 and Hsp27) or in both the cytoplasm and the nucleus (Hsp110, Hsp70, and Hsp40), except Hsp60 that shows mitochondria staining pattern (Fig. 1B, c-n, e'-n'). We next examined Hsp expression in HSF1-null epidermis, and found that staining intensity and localization of Hsp stainings are similar in both wild-type and HSF1-null epidermis. These results indicate that HSF1 is not involved in expression of major Hsps in SDS-treated epidermal cells, and suggest that chronic cutaneous inflammation and irritation may not induce heat shock response.

Induction of Hsp70 by heat shock is mediated by activation of HSF1 *in vivo* in the mouse skin

To examine whether Hsps are induced by heat shock in this model, the skin of SDS-treated mice was heat-treated at 50℃ for 3 min and expression of major heat-inducible Hsp70 was examined. Heat treatment greatly increased Hsp70 staining dominantly in the nucleus in all the layers of the HSF1+/ - epidermis (Fig. 2A) and of the wild-type epidermis (data not shown), as was shown in the

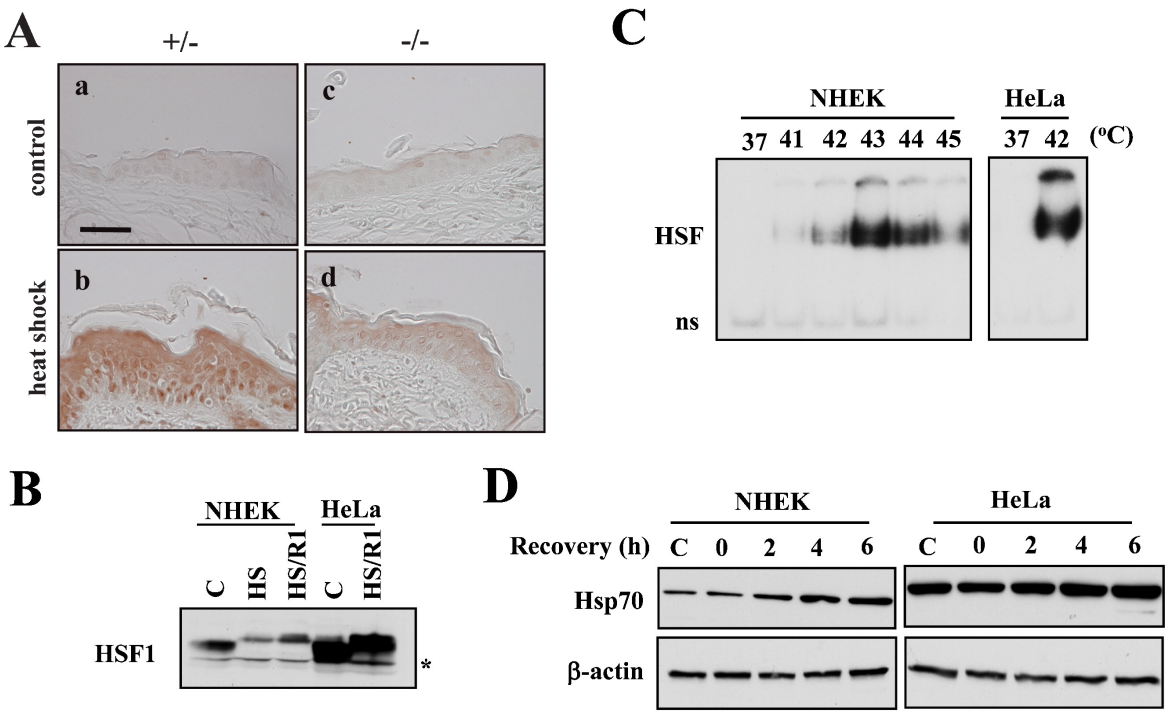

Fig. 2 HSF1 is required for induction of Hsp70 in vivo in the mouse epidermis.
A, Anesthetized SDS-treated untreated (control) and heat-shocked (heat shock) skin of heterozygous (HSF1+/-) and HSF1-null (HSF1-/-) mice were treated at 50 ℃ for 3 min, and then allowed to recover for 3 hr. Skins were dissected, fixed, and embedded in paraffin. Sections (4μm in thickness) were stained with anti-Hsp70 antibody. Developing time was much shorted here than in Fig. 1. B, Western blot analysis of HSF1 was performed on NHEK and HeLa cells after heat shock at 43 ℃ for 30 min (HS) with recovery time of 1 hr (HS/R1). The asterisk indicates non-specific bands. C, Gel shift assay using extracts isolated from NHEK and HeLa cells treated with heat shock for 30 min at indicated temperatures. D, Western blot analysis of Hsp70 in NHEK and HeLa cells treated with or without (C) heat shock at 43℃ for 30 min at indicated recovery times.

human skin.[12)13)] In contrast, Hsp70 staining was hardly increased under the heat-primed condition in HSF1-null epidermis. To examine whether HSF1 is activated in keratinocytes in response to heat shock like in other cells, we used cultures of normal human epidermal keratinocytes (NHEK). In unstressed cells HSF1 is present mostly as a monomer and is converted to a trimer that binds to heat shock element (HSE) in response to heat shock. HSF1 in NHEK cells was hyperphosphorylated and acquired HSE-binding activity by heat shock at 43℃ like that in HeLa cells (Fig. 2B, C). Hsp70 was also heat-induced in NHEK cells (Fig. 2D).[11)] These results suggest that Hsp70 induction is mediated by *in vivo* activation of HSF1 in the mouse skin as well as human skin.

More weakness against heat was seen in HSF1-null than wild-type mouse

To examine whether HSF1 is important for resistance of the epidermis against heat, SDS-treated skin of anesthetized wild type and heterozygous (HSF1+/-) and HSF1-null (-/-) mice were exposed to various temperature and time of heat treatment to determine the effects on morphological changes. Clear morphological changes between these mice were seen 3 hours after heat shock at 50℃ for 3 min (Fig. 3A). In HSF1-null epidermis, nuclei of basal cells appear pyknotic and possess a perinuclear halo, or show slight eosinophilic staining. In contrast, the wild type and heterozygous (HSF1+/-) epidermis had less morphological changes. Consistently, subepidermal blister was much less observed in the wild type and heterozygous (HSF1+/-) (Fig. 3B). These results indicate that HSF1 is important for protection of epidermal cells against heat.

Discussion

We showed that Hsps are highly expressed in unstressed mouse epidermis independently of HSF1, and HSF1 is required for further induction of Hsps under heat shock condition. It is well known that Hsps protect cellular proteins from denaturation and aggregate formation under heat shock conditions.[19)] Furthermore, Hsps inhibit proapoptotic factors such as Apaf-1 and cytochrome c.[20-24)]

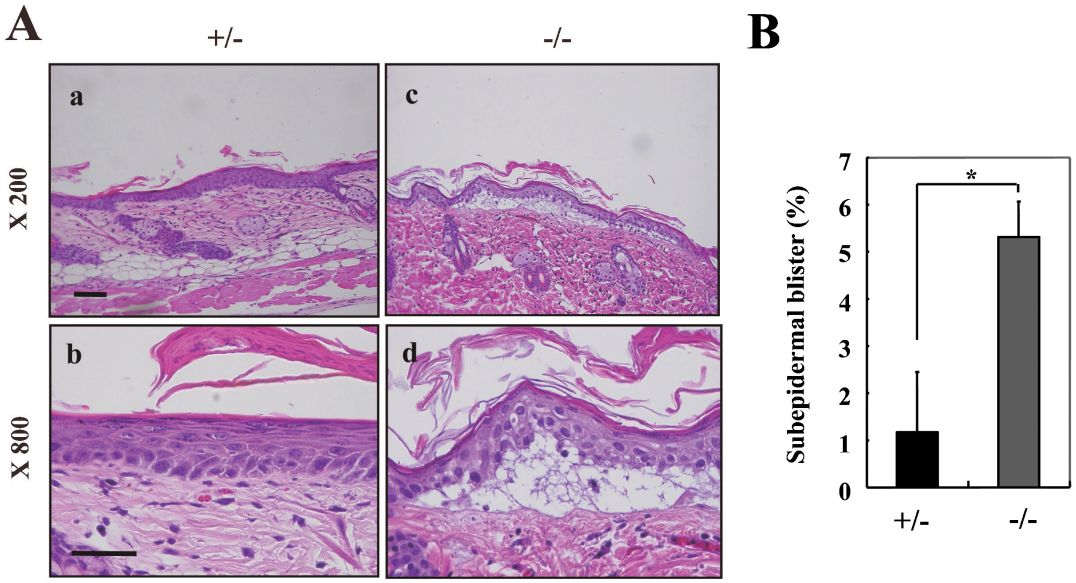

Fig. 3 HSF1 is required for protection of the epidermis against heat shock.
A, SDS-treated skin of anesthetized heterozygous (HSF1+/-) and HSF1-null (-/-) mice was placed on a hot plate for heat shock at 50℃ for 3 min, and then allowed to recover for 3 hr. The skins were dissected, fixed, and embedded in paraffin. Sections (4μm in thickness) were stained with hematoxylin and eosin. Magnitude, x 200 (a, c) and x 800 (b, d). B, Percentages of skin region having subepidermal blister (n=3). The asterisk indicates $p < 0.05$.

Moreover, we previously showed that HSF1 protects from cell death through regulating unknown target genes in addition to the regulation of Hsp expression.[5)25)] These observations strongly suggest a biologically protective roles of HSF1. In contrast, we showed a pro-apoptotic role of HSF1 by inducing a pro-apoptotic *TDAG51* gene.[26)] In fact, germ cells are more sensitive to high temperatures in the presence of HSF1.[27)28)] However, we have showed that HSF1 plays a protective role in the mouse epidermis exposed to a high temperature (50℃) by using HSF1-null mice data.

It was shown previously that Hsps are expressed in unstressed human skin, and is also induced by heat shock.[12)13)15)] However, there is no report that shows clear induction of Hsps in the mouse epidermis by *in vivo* heat shock. There are two difficulties for the analysis of Hsps in the mouse epidermis. First, we do not know temperatures that induce Hsps or damage epidermal cells. We found that Hsps are little induced in the epidermis when mice were submerged in a water bath at 44℃ for 5 min (data not shown). Longer exposure of mice at this temperature results in death of the mice. Therefore, we used local hyperthermia at 50℃ for 3 min by using a hot plate. Finally, we did show clear induction of Hsps in the mouse epidermis.

Second, the epidermis of adult mice is too thin to detect intensity and localization of Hsp staining. To clarify localization of Hsps and to examine differentiation-related Hsp expression, we used an *in vivo* model of chronic cutaneous inflammation and irritation. As this treatment itself did not induce heart shock response, we were able to analyze expression of Hsps in proliferated epidermal cells. We found that Hsp70 and Hsp27 expression increased as distance of keratinocytes from the basal layer increased, and was in parallel with the extent of keratinization. Although Hsp70 and Hsp27 induction could be associated with cell differentiation of keratinocytes upon the inflammatory skin conditions, these observations are consistent with previous report about Hsp27 induction during embryonic mouse development or during keratinocyte differentiation.[16)17)] Hsp27 is associated with cytoskeletal proteins and filaggrin that is important for keratinization.[18)] In addition to Hsp27, we showed here that Hsp70 expression was associated with keratinocyte differentiation independently of HSF1. As keratinocyte differentiation involves dynamic processes including keratinization,[29)] Hsp70 and Hsp27 molecular chaperones may facilitate these processes. Various inflammatory and hereditary diseases such as psoriasis and ichthyoses are characterized with disturbance of keratinocyte differentiation. Especially in psoriasis, Hsp70 of mouse keratinocyte which is a ligand for CD91 receptor interacts CD91-positive dermal dendritic antigen-presenting cells, leading to production of TNF-α, an important pro-inflammatory cytokine in the development of psoriasis.[30)] Exploration of this issue using the skin irritation mouse model as an analytical tool comes to the idea of therapeutic modification of Hsp expression through HSF1.

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