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Short Communication

Ultraviolet A Induces Endoplasmic Reticulum Stress Response in Human Dermal Fibroblasts

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ABSTRACT. **The endoplasmic reticulum (ER) stress response is a cytoprotective mechanism against the accumulation of unfolded proteins in the ER (ER stress) that consists of three response pathways (the ATF6, IRE1 and PERK pathways) in mammals. These pathways regulate the transcription of ER-related genes through specific cis-acting elements, ERSE, UPRE and AARE, respectively. Because the mammalian ER stress response is markedly activated in professional secretory cells, its main function was thought to be to upregulate the capacity of protein folding in the ER in accordance with the increased synthesis of secretory proteins. Here, we found that ultraviolet A (UVA) irradiation induced the conversion of an ER-localized sensor pATF6α(P) to an active transcription factor pATF6α(N) in normal human dermal fibroblasts (NHDFs). UVA also induced IRE1-mediated splicing of *XBP1* mRNA as well as PERK-mediated phosphorylation of an α subunit of eukaryotic initiation factor 2. Consistent with these observations, we found that UVA increased transcription from ERSE, UPRE and AARE elements. From these results, we concluded that UVA irradiation activates all branches of the mammalian ER stress response in NHDFs. This suggests that the mammalian ER stress response is activated by not only intrinsic stress but also environmental stress.**

Key words: ER stress/ultraviolet A/ATF6/XBP1/PERK

Introduction

Endoplasmic reticulum (ER) stress is a harmful condition, in which the folding of secretory proteins is hampered and unfolded proteins are accumulated in the ER. Upon the occurrence of ER stress, eukaryotic cells activate a cytoprotective mechanism called the ER stress response (Kohno, 2010; Mori, 2010; Ron and Walter, 2007; Scheuner and Kaufman, 2008; Yoshida, 2007), which is conserved from yeast to mammals.

The mammalian ER stress response consists of three signaling pathways, the first of which is the ATF6 pathway. Upon ER stress, pATF6(P), a transmembrane protein located in the ER, is converted from an oligomer to a monomer by the reduction of disulfide bonds between the monomers. These monomers are transported to the Golgi apparatus and cleaved by Golgi-localized proteases. The cytosolic fragment of cleaved ATF6 (pATF6(N)) translocates to the nucleus, binds to an enhancer element called ERSE, and activates the transcription of genes encoding ER chaperones in order to enhance the folding of secretory proteins. The second pathway is the IRE1 pathway. IRE1 located in the ER membrane is activated by ER stress and converts the pre-mRNA of *XBP1* (*XBP1(U)* mRNA) to the mature mRNA (*XBP1(S)* mRNA) by unconventional mRNA splicing. pXBP1(S), an active transcription factor, is translated from *XBP1(S)* mRNA and activates the transcription of genes encoding ER-associated degradation (ERAD) components through an enhancer element called UPRE, in order to enhance the degradation of unfolded or malfolded secretory proteins accumulated in the ER. The third pathway is the PERK pathway. When the transmembrane kinase PERK is activated by ER stress, it phosphorylates the α subunit of eukaryotic initiation factor (eIF2α), resulting in translational attenuation in order to halt the further accumu-

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lation of unfolded proteins in the ER. PERK also increases the expression of a transcription factor, ATF4, which binds to an enhancer element called AARE and enhances the transcription of CHOP and GADD34, a proapoptotic transcription factor and a phosphatase specific to eIF2α, respectively. Because the mammalian ER stress response is markedly activated in professional secretory cells, including pancreatic beta cells, acinar cells in the salivary gland, and plasma cells (Lee *et al.*, 2005; Reimold *et al.*, 2001), it has been thought that the main function of the mammalian ER stress response is to increase the capacity of protein folding in the ER when the synthesis of secretory proteins is increased. However, cells other than professional secretory cells also exhibit the ER stress response system, suggesting that the ER stress response is activated in other situations in order to protects cells from stressful environments.

Ultraviolet (UV) irradiation is a harmful electromagnetic wave that has become an increasingly serious issue because of the gradual thinning of the earth's ozone layer. UV is usually classified into three categories, UVA (wavelength 315–400 nm), UVB (280–315 nm) and UVC (200–280 nm). UVA damages both the epidermis and the dermis, whereas UVB affects only the epidermis. UVC hardly reaches human skin because it is absorbed by the ozone layer; therefore, UVA is thought to be one of the major factors responsible for photo-induced damage of the dermis. In this article, we investigated whether UVA irradiation induces ER stress in normal human dermal fibroblasts (NHDFs), to investigate whether the mammalian ER stress response is activated by not only intrinsic stress but also environmental stress.

Materials and Methods

Cell culture and transfection

NHDFs cells were cultured as a culture of HeLa cells (Yoshida *et al.*, 2001). Cells were irradiated with UVA in phosphate-buffered saline (PBS), incubated in DMEM-FCS for the indicated time period after UVA irradiation and then harvested for immunoblotting or RT-PCR.

Immunoblotting and RT-PCR

Immunoblotting (Yoshida *et al.*, 2003) and RT-PCR of *XBP1* mRNA (Uemura *et al.*, 2009) were performed essentially as described previously. For immunoblotting, recycled anti-ATF6α antiserum used in other studies was reused. For RT-PCR, total RNA was reverse-transcribed using Superscript VILO reverse transcriptase (Invitrogen) and amplified with MightyAmp DNA polymerase (TaKaRa) using a pair of primers that correspond to nucleotides 493–512 (CGCGGATCCGAATGAAGTGAGGCCAGTGG) and 834–853 (GGGGCTTGGTATATATGTGG) of *XBP1* mRNA, respectively. Amplified fragments covering a 26-nt intron (nucleotides 531–556), and flanking exon fragments were separated on TAE-polyacrylamide gels, visualized by staining with Gel Red (Biotium) and detected using an LAS-3000 (Fuji Film).

Luciferase assay

Transient transfection of HeLa cells was carried out as described previously (Oku *et al.*, 2011). In brief, HeLa cells cultured in a 24-well plate were incubated with precipitates of calcium phosphate containing plasmids for 12 h at 37°C. After washing with PBS to remove $CaPO_4$-DNA precipitates, cells were incubated in fresh medium for 24 h, irradiated with UVA as described above and harvested for luciferase assays. Construction of the ERSE, UPRE and AARE reporter plasmids was described previously (Wang *et al.*, 2000; Yoshida *et al.*, 1998; Yoshida *et al.*, 2000).

Results and Discussion

We examined the spectral profile of the UVA source used here and confirmed that it was within the range of 315–400 nm (Fig. 1). We first examined whether UVA irradiation (315–400nm) activated the ATF6 pathway. Whole cell lysates prepared from NHDFs irradiated with UVA were subjected to immunoblotting analysis using an anti-ATF6α antibody. It was found that 0.2–10 J/cm^2 UVA induced the cleavage of pATF6α(P) and expression of pATF6α(N) (Fig. 2A, lanes 4–9), as well as an authentic ER stress inducer, thapsigargin (lane 10). Because the total dose of UVA irradiation during daytime around the Tokyo area in July is 150 J/cm^2, and the penetration rate of UVA (350 nm) at the depth of 120 μm in human skin is 14% (Anderson and Parrish, 1981), the UVA dose used here is physiologically significant. Expression of pATF6α(N) diminished considerably in cells irradiated with 5 or 10 J/cm^2 UVA (lanes 8 and 9). It is possible that excessive oxidative stress caused by strong UVA irradiation over-oxidized pATF6α(P), resulting in retention of pATF6α(P) in the ER lumen because reduc-

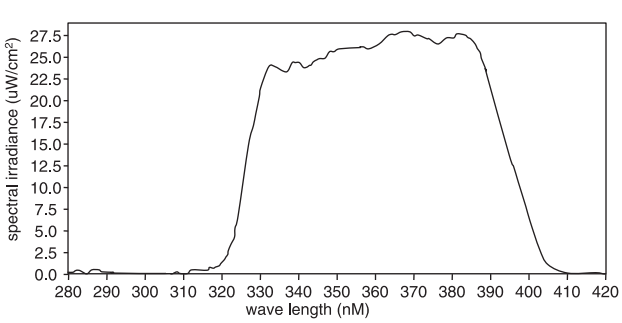

Fig. 1. Confirmation of the wavelength of the UVA source. The spectral profile of the UVA source is shown. UVA was delivered using a Xenon lamp through a UVA-specific bandpass filter (Asahi Spectra Co.). The profile was measured using a MSR-7000 (Opt Research Inc.). Experimental exposure time needed to deliver 1 J/cm^2 of UVA was 10.95 sec.

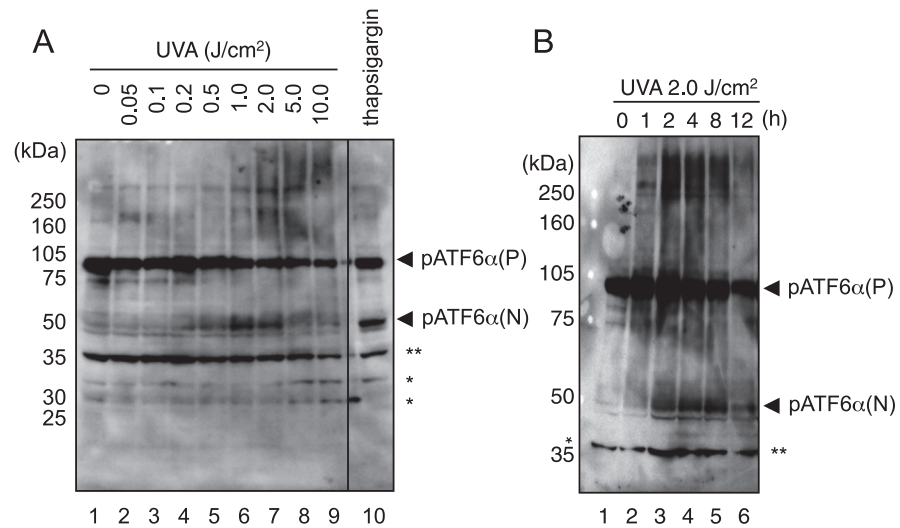

Fig. 2. Immunoblotting analyses of the ATF6 pathway after UVA irradiation. Whole cell lysates prepared from NHDFs (A) irradiated with the indicated dose of UVA and incubated in growth medium for 6 h before harvest or (B) irradiated with 2.0 J/cm² UVA and incubated in growth medium for the indicated periods were separated by SDS-PAGE, blotted onto a nylon membrane, and probed with an anti-ATF6α antibody. Asterisks indicate non-specific bands. Non-specific bands indicated by double asterisks were used as a loading control.

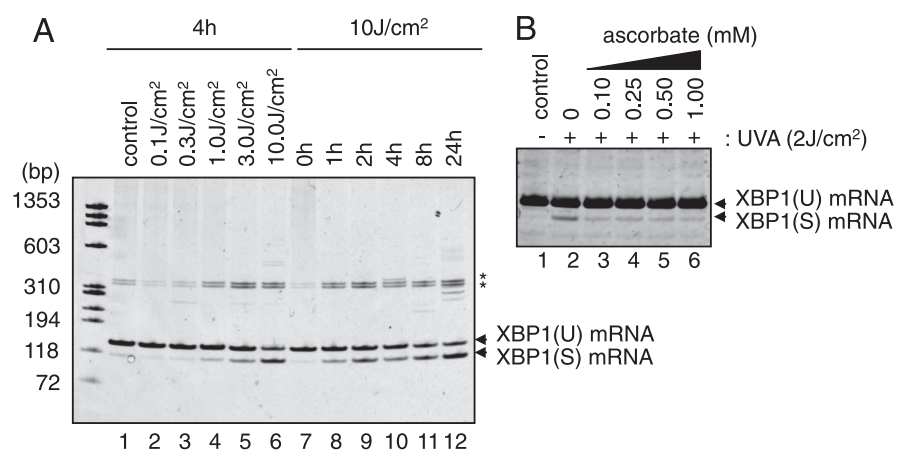

Fig. 3. RT-PCR analyses of the XBP1 pathway after UVA irradiation. (A) Total RNA prepared from NHDFs irradiated with the indicated dose of UVA and incubated in growth medium for the indicated periods after UVA irradiation was used as a template for RT-PCR to amplify the region of *XBP1* mRNA splicing. RT-PCR products were separated on HCHO-polyacrylamide gel and stained with Gel Red. (B) *XBP1* splicing in NHDFs treated with the indicated dose of ascorbate and UVA was examined as in (A).

tion of intermolecular disulfide bonds between pATF6α(P) is prerequisite for the conversion of pATF6α(P) to pATF6α(N) (Nadanaka *et al.*, 2007). UVA-induced cleavage of pATF6α(P) was observed at 2–12 hours after 2.0 J/cm² UVA irradiation (Fig. 2B, lanes 3–6), indicating that UVA activated the ATF6 pathway.

Next, the IRE1 pathway was examined. Total RNA prepared from UVA-irradiated NHDFs was subjected to RT-PCR to examine *XBP1* splicing (Fig. 3A). Before UVA irradiation, only *XBP1(U)* mRNA was observed (lanes 1 and 7). After 1–10 J/cm² UVA irradiation, *XBP1(S)* mRNA levels were increased (lanes 4–6 and 8–12), indicating that the IRE1 pathway was activated by UVA irradiation and the splicing of *XBP1* mRNA was induced. This UVA-induced splicing of *XBP1* mRNA was suppressed by treatment with an antioxidant, ascorbate (Fig. 3B), suggesting that oxidative stress is involved in the induction of ER stress by UVA irradiation.

We also examined whether UVA irradiation activated the PERK pathway. Whole cell lysates prepared from UVA-irradiated NHDFs were subjected to immunoblotting analysis using anti-phosphorylated eIF2α (upper panel in

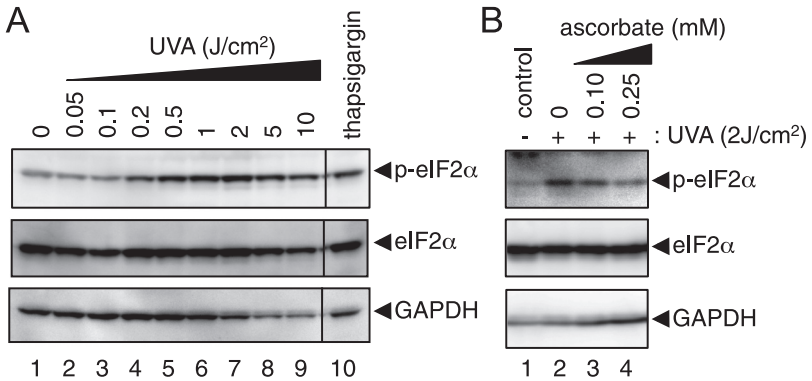

Fig. 4. Analysis of the PERK pathway after UVA irradiation. The same samples as in Fig. 2A was subjected to immunoblotting and probed with anti-phosphorylated eIF2α (upper panel), anti-eIF2α (middle panel) and anti-GAPDH (lower panel) antibodies.

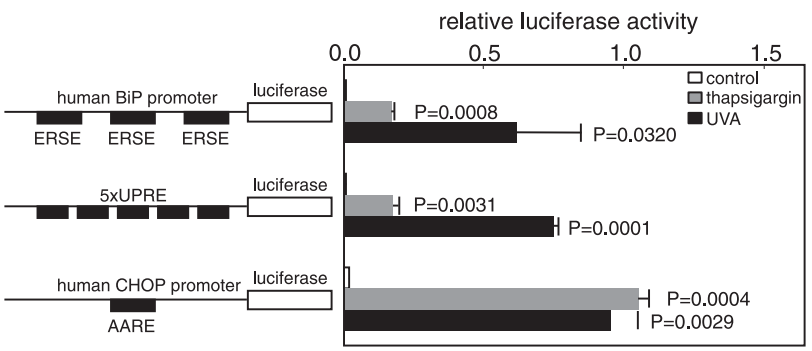

Fig. 5. Analysis of transcription from ERSE, UPRE and AARE after UVA irradiation. NHDFs were transiently transfected with the indicated reporter plasmids and a Renilla luciferase standard plasmid. After 1μM thapsigargin treatment (grey bars) or 2.0 J/cm^2 UVA irradiation (black bars), cells were harvested for luciferase assays. Luciferase activity was corrected with the internal standard Renilla luciferase activity.

Fig. 4A), anti-eIF2α (middle panel) and anti-GAPDH (lower panel) antibodies. Phosphorylation of eIF2α a target of PERK, was increased by UVA irradiation (upper panel, lanes 1–9) as well as after thapsigargin treatment (lane 10), whereas the expression of eIF2α (middle panel) and GAPDH used as a loading control was not (lower panel). This UVA-induced phosphorylation of eIF2α was again suppressed by treatment with ascorbate (Fig. 4B, lanes 3 and 4). We did not observed suppression of the UVA-induced cleavage of pATF6(P) by ascorbate treatment (data not shown), and it was speculated that this was because ascorbate enhanced the conversion of PATF6(P) oligomers to monomers by hampering oxidative conditions in the ER.

Finally, we examined whether transcription from ERSE, UPRE and AARE was induced by UVA irradiation. Reporter constructs harboring a firefly luciferase gene fused with ERSE, UPRE or AARE were transiently transfected into NHDFs, and luciferase activity was measured after UVA irradiation or thapsigargin treatment. UVA irradiation enhanced transcription from these ER stress-responsive cis-acting elements as well as thapsigargin (Fig. 5). From these results, we concluded that UVA irradiation induces the ER stress response in NHDFs. This suggests that the ER stress response is activated throughout mammalian skin in response to UVA irradiation, and that the ER stress response is activated by not only intrinsic stress (increased synthesis of secretory proteins) but also extrinsic environmental stress.

Below we present our working hypothesis on UVA-induced activation of the ER stress response in human dermal cells. UVA irradiation causes oxidative stress and produces reactive oxygen species (ROS), including superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, singlet oxygen, and lipid peroxides and their radicals (Gruber *et al.*, 2010; Sakurai *et al.*, 2005; Tyrrell, 2004). ROS affects protein folding in the ER and finally induces ER stress (Malhotra and Kaufman, 2007; Zhang, 2010). Under these ER stress conditions, the folding of ECM proteins such as collagen and elastin might be impaired, possibly resulting in their enhanced retention in the ER as well as degradation by ERAD. Reduced expression of ECM proteins in the extracellular matrix might lead to acceleration of the aging process of dermal skin. The importance of the ER stress response in protection against photo-induced

skin damage in humans will be of interest for future research.

It is interesting that the dose that each of the UPR branches responded to UVA was different. Each branch of the UPR might respond to distinct molecular changes in the ER caused by ER stress. It is possible that ATF6 could not be activated by higher doses of UVA because the activation machinery of the pathway is labile to oxidative stress caused by UVA, as mentioned above. Moreover, the PERK pathway and the oxidative stress response pathway are known to be interconnected (Cullinan *et al.*, 2003) and this may contribute to the high sensitivity of the PERK pathway to UVA irradiation.

Previously, Kaufman and colleagues reported that UVC (254 nm) activates the PERK pathway in COS-1, MCF-7 and HIT cells (Wu *et al.*, 2002), and Maytin and colleagues showed that UV light consisting of 74% UVB (280–320 nm) and 16% UVA induced the transcription of CHOP in murine epidermis (Anand *et al.*, 2005). Recently, Kanekura and colleagues revealed that UVB (around 302 nm) activated the ATF6 and IRE1 pathways, but not the PERK pathway in human keratinocytes (Mera *et al.*, 2010); however, to the authors' knowledge, this is the first report demonstrating that UVA (315–400 nm) activates the ER stress response in eukaryotic cells.

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References

Anand, S., Chakrabarti, E., Kawamura, H., Taylor, C.R., and Maytin, E.V. 2005. Ultraviolet light (UVB and UVA) induces the damage-responsive transcription factor CHOP/gadd153 in murine and human epidermis: evidence for a mechanism specific to intact skin. *J. Invest. Dermatol.*, **125**: 323–333.

Anderson, R.R. and Parrish, J.A. 1981. The optics of human skin. *J. Invest. Dermatol.*, **77**: 13–19.

Cullinan, S.B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R.J., and Diehl, J.A. 2003. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell. Biol.*, **23**: 7198–7209.

Gruber, F., Mayer, H., Lengauer, B., Mlitz, V., Sanders, J.M., Kadl, A., Bilban, M., de Martin, R., Wagner, O., Kensler, T.W., Yamamoto, M., Leitinger, N., and Tschachler, E. 2010. NF-E2-related factor 2 regulates the stress response to UVA-1-oxidized phospholipids in skin cells. *FASEB J.*, **24**: 39–48.

Kohno, K. 2010. Stress-sensing mechanisms in the unfolded protein response: similarities and differences between yeast and mammals. *J. Biochem.*, **147**: 27–33.

Lee, A.H., Chu, G.C., Iwakoshi, N.N., and Glimcher, L.H. 2005. XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J.*, **24**: 4368–4380.

Malhotra, J.D. and Kaufman, R.J. 2007. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signal.*, **9**: 2277–2293.

Mera, K., Kawahara, K., Tada, K., Kawai, K., Hashiguchi, T., Maruyama, I., and Kanekura, T. 2010. ER signaling is activated to protect human HaCaT keratinocytes from ER stress induced by environmental doses of UVB. *Biochem. Biophys. Res. Commun.*, **397**: 350–354.

Mori, K. 2010. Divest yourself of a preconceived idea: transcription factor ATF6 is not a soluble protein! *Mol. Biol. Cell*, **21**: 1435–1438.

Nadanaka, S., Okada, T., Yoshida, H., and Mori, K. 2007. Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. *Mol. Cell. Biol.*, **27**: 1027–1043.

Oku, M., Tanakura, S., Uemura, A., Sohda, M., Misumi, Y., Taniguchi, M., Wakabayashi, S., and Yoshida, H. 2011. Novel cis-acting element GASE regulates transcriptional induction by the Golgi stress response. *Cell Struct. Funct.*, **36**: 1–12.

Reimold, A.M., Iwakoshi, N.N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E.M., Friend, D., Grusby, M.J., Alt, F., and Glimcher, L.H. 2001. Plasma cell differentiation requires the transcription factor XBP-1. *Nature*, **412**: 300–307.

Ron, D. and Walter, P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.*, **8**: 519–529.

Sakurai, A., Nishimoto, M., Himeno, S., Imura, N., Tsujimoto, M., Kunimoto, M., and Hara, S. 2005. Transcriptional regulation of thioredoxin reductase 1 expression by cadmium in vascular endothelial cells: role of NF-E2-related factor-2. *J. Cell. Physiol.*, **203**: 529–537.

Scheuner, D. and Kaufman, R.J. 2008. The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. *Endocr. Rev.*, **29**: 317–333.

Tyrrell, R.M. 2004. Solar ultraviolet A radiation: an oxidizing skin carcinogen that activates heme oxygenase-1. *Antioxid. Redox Signal.*, **6**: 835–840.

Uemura, A., Oku, M., Mori, K., and Yoshida, H. 2009. Unconventional splicing of XBP1 mRNA occurs in the cytoplasm during the mammalian unfolded protein response. *J. Cell Sci.*, **122**: 2877–2886.

Wang, Y., Shen, J., Arenzana, N., Tirasophon, W., Kaufman, R.J., and Prywes, R. 2000. Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. *J. Biol. Chem.*, **275**: 27013–27020.

Wu, S., Hu, Y., Wang, J.L., Chatterjee, M., Shi, Y., and Kaufman, R.J. 2002. Ultraviolet light inhibits translation through activation of the unfolded protein response kinase PERK in the lumen of the endoplasmic reticulum. *J. Biol. Chem.*, **277**: 18077–18083.

Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. 1998. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J. Biol. Chem.*, **273**: 33741–33749.

Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. 2000. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol. Cell. Biol.*, **20**: 6755–6767.

Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. 2001. Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response. *Mol. Cell Biol.*, **21**: 1239–1248.

Yoshida, H., Matsui, T., Hosokawa, N., Kaufman, R.J., Nagata, K., and Mori, K. 2003. A time-dependent phase shift in the mammalian unfolded protein response. *Dev. Cell*, **4**: 265–271.

Yoshida, H. 2007. ER stress and diseases. *FEBS J.*, **274**: 630–658.

Zhang, K. 2010. Integration of ER stress, oxidative stress and the inflammatory response in health and disease. *Int. J. Clin. Exp. Med.*, **3**: 33–40.

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