Biochemical and Biophysical Research Communications 409 (2011) 90–95

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Characterization of stress sensitivity and chaperone activity of Hsp105 in mammalian cells

Nobuyuki Yamagishi [a], Masayasu Yokota [a], Kunihiko Yasuda [b], Youhei Saito [a], Kazuhiro Nagata [c], Takumi Hatayama [a,*]

[a] Department of Biochemistry & Molecular Biology, Division of Biological Sciences, Kyoto Pharmaceutical University, Japan
[b] Department of Anatomy and Neurobiology, Unit of Basic Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Japan
[c] Laboratory of Molecular and Cellular Biology, Faculty of Life Sciences, Kyoto Sangyo University, Japan

ARTICLE INFO

Article history:
Received 30 March 2011
Available online 1 May 2011

Keywords:
Hsp105
Apoptosis
Chaperone activity
Luciferase

ABSTRACT

Hsp105 is a major mammalian heat shock protein that belongs to the Hsp105/110 family, a diverged subgroup of the Hsp70 family. Hsp105 not only protects the thermal aggregation of proteins, but also regulates the Hsc70 chaperone system *in vitro*. Recently, it has been shown that Hsp105/110 family members act as nucleotide exchange factors for cytosolic Hsp70s. However, the biological functions of Hsp105/110 family proteins still remain to be clarified. Here, we examined the function of Hsp105 in mammalian cells, and showed that the sensitivity to various stresses was enhanced in the Hsp105-deficient cells compared with that in control cells. In addition, we found that deficiency of Hsp105 impaired the refolding of heat-denatured luciferase in mammalian cells. In contrast, overexpression of Hsp105α enhanced the ability to recover heat-inactivated luciferase in mammalian cells. Thus, Hsp105 may play an important role in the refolding of denatured proteins and protection against stress-induced cell death in mammalian cells.
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1. Introduction

Heat shock proteins are a set of highly conserved proteins produced in response to physiological and environmental stresses that serve to protect cells from stress-induced damage by preventing protein denaturation and/or repairing such damage [1]. Mammalian heat shock proteins are classified into several families on the basis of their apparent molecular weight and function, such as HSP105/110, HSP90, HSP70, HSP60, HSP40, and HSP27. The HSP 70 family is the major and best-characterized group of heat shock proteins. Several different species of HSP70 family proteins are present in different compartments of eukaryotic cells and play important roles as molecular chaperones that prevent the irreversible aggregation of denatured proteins. Hsp70s also assist the folding, assembly, and translocation across the membrane of cellular proteins [2,3].

Hsp105α and Hsp105β are mammalian members of the Hsp105/110 family, a diverged subgroup of the Hsp70 family. Hsp105α is expressed constitutively and induced by various forms of stress, while Hsp105β is an alternatively spliced form of Hsp105α that is specifically produced following heat shock at 42 °C [4–6]. These proteins suppress the aggregation of denatured proteins caused by heat shock *in vitro*, as does Hsp70 [7]. Furthermore, Hsp105α and Hsp105β exist as complexes associated with Hsp70 and Hsc70 in mammalian cells [8], and regulate the Hsc70 chaperone system [7,9]. Recently, it has been shown that mammalian and yeast Hsp105/110 family members act as nucleotide exchange factors for cytosolic Hsp70s [10–12]. Furthermore, the yeast Hsp105/110 homolog, Sse1p, has been shown to be an important component of the folding machinery for newly synthesized proteins [10] and heat-denatured proteins [11]. However, human and yeast Hsp105/110 proteins show significant differences in their biochemical properties, such as thermostability and intrinsic ATPase activity [13]. In addition, several evolutionarily unrelated families of NEFs have been identified in mammalian cells, and it is unclear whether mammalian Hsp105/110 family proteins act as an important component of the protein folding machinery in mammalian cells.

Here, we examine the function of Hsp105α in mammalian cells using the Hsp105-deficient cells, and show that Hsp105α plays an important role in protein refolding and protection against stress-induced cell death in mammalian cells.

2. Materials and methods

2.1. Cell culture and stress treatments

Mouse fibroblast C3H10T1/2 cells were obtained from RIKEN Bioresource Center Cell Bank (Tsukuba, Japan) and were cultured

* Corresponding author. Address: Department of Biochemistry & Molecular Biology, Division of Biological Sciences, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan. Fax: +81 75 595 4758.
E-mail address: hatayama@mb.kyoto-phu.ac.jp (T. Hatayama).

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doi:10.1016/j.bbrc.2011.04.114

in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 in air at 37 °C. Hsp105-deficient mouse embryonic fibroblasts (*hsp105*$^{-/-}$ MEFs) and wild-type mouse embryonic fibroblasts (*hsp105*$^{+/+}$ MEFs) were prepared from day 13.5 hsp105 knockout mouse embryos as described previously [14]. These cells were cultured in DMEM containing a higher concentration of glucose (4.5 mg/ml) supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 in air at 37 °C.

Staurosporine was dissolved in dimethylsulfoxide at a concentration of 1 mM, and diluted with culture medium to concentrations ranging from 0.05 to 0.8 μM. Cells were incubated in the

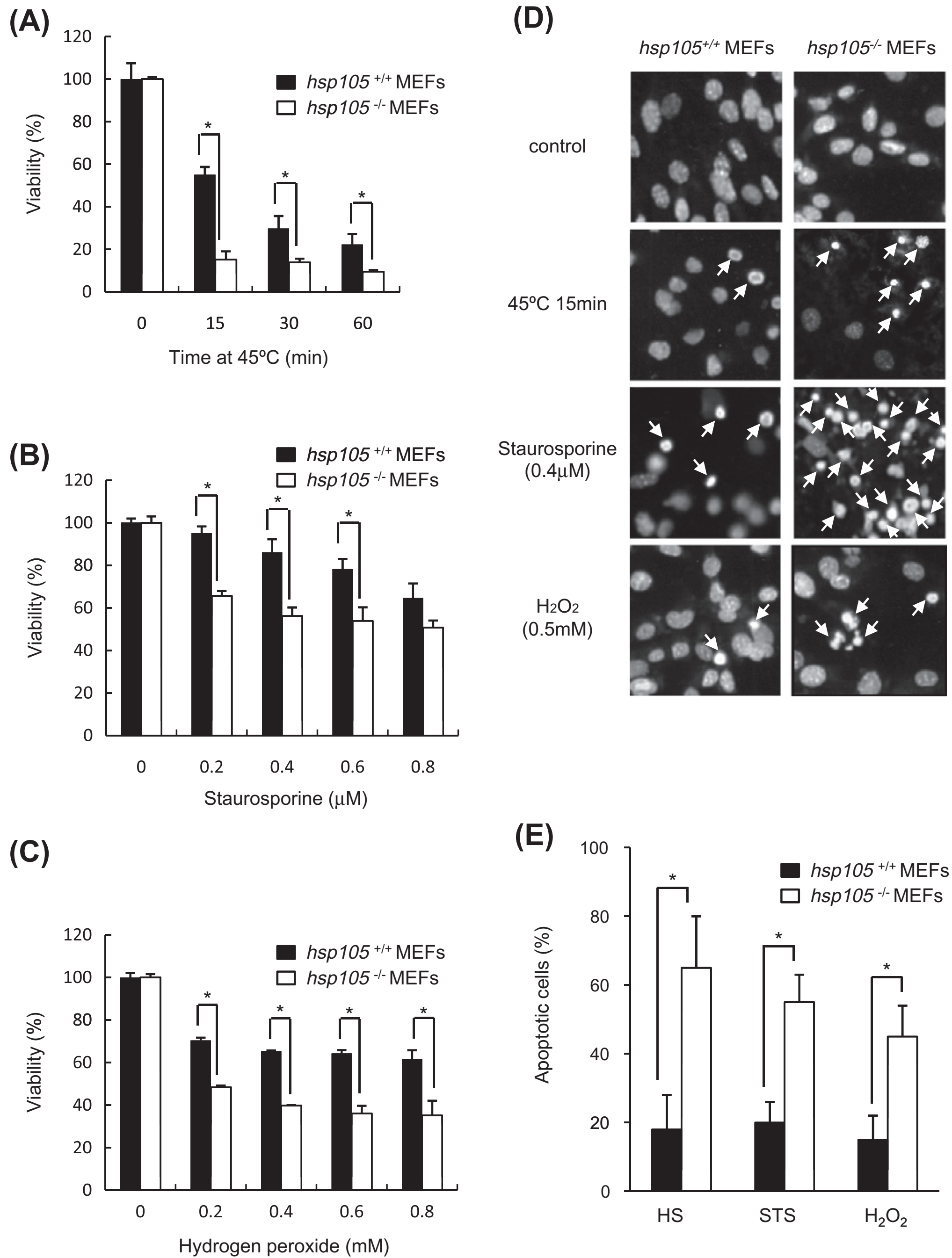

Fig. 1. Deficiency of Hsp105 enhances the sensitivity to various stresses in mouse embryonic fibroblasts. Wild-type (*hsp105*$^{+/+}$) MEFs and *hsp105*$^{-/-}$ MEFs were treated at 45 °C for 15–60 min and further incubated at 37 °C for 6 h (A), exposed to 0.2–0.8 μM staurosporine (B), or exposed to 0.2–0.8 mM H_2O_2 for 1 h and further incubated at 37 °C for 6 h (C). Cell viability was then determined by neutral red assay. Values represent the means ± SD of three independent experiments performed in triplicate. The significance of differences was assessed by an unpaired Student's *t*-test. $^*p < 0.05$ for viability in *hsp105*$^{+/+}$ MEFs vs. *hsp105*$^{-/-}$ MEFs. (D) *hsp105*$^{+/+}$ MEFs and *hsp105*$^{-/-}$ MEFs cells were grown on coverslips, treated at 45 °C for 15 min and further incubated at 37 °C for 6 h, exposed to 0.4 μM staurosporine for 6 h, or exposed to 0.5 mM H_2O_2 for 1 h and further incubated at 37 °C for 6 h. Cells were washed with PBS, fixed with 3.7% formaldehyde, and stained with 10 μM Hoechst 33342. Nuclear morphology of cells was observed using a fluorescence microscope. Arrows indicate the apoptotic cells. (E) Rates of apoptosis were calculated using at least 200 cells in each experiment. Values represent the means ± SD of four independent experiments. The significance of differences was assessed with an unpaired Student's *t*-test. $^*p < 0.01$ for viability in *hsp105*$^{+/+}$ MEFs vs. *hsp105*$^{-/-}$ MEFs.

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