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Intracellular Localization of Heat Shock Proteins in a Rat Hepatoma Cell Line

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ラット肝癌由来細胞H-4-II-Eにおける熱ショックタンパク質の細胞内局在

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Abstract

The highly conserved response of all cell types and organisms to heat and other forms of physiological stress has been well characterized. In this study, we focused on the different types of heat shock proteins (Hsp), Hsp32/heme oxygenase-1 (HO-1) and Hsp70, and investigated their intracellular localization in rat hepatoma cell line (H-4-II-E) after induction by stress loading, using confocal laser scanning microscope. When cells were treated with hemin, which was strong inducer of HO-1, marked induction of HO-1 was observed after 6 hr. Intracellular localization of HO-1 was restricted in endoplasmic reticulum after induction as well as untreated control. We observed that the induction level of HO-1 was almost the same between cells. On the other hand, the distribution of Hsp70 after heat shock loading (HS) was unique. Hsp70 localized mainly in cytosol, moved into nucleus immediately after HS, then it was markedly induced in cytosol after 6 hr. In addition, induction level of Hsp70 varied extensively among cells. These observations indicated that Hsps localized in the right place to show appropriate function, according to the cell condition.

Keywords: heat shock protein, heme oxygenase-1, Hsp70, intracellular localization

Introduction

Heme oxygenase (HO) catalyze the conversion of heme to carbon monoxide and bilirubin with a concurrent release of iron. HO is the sole physiological pathway of heme degradation and, consequently, plays a critical role in the regulation of cellular heme-dependent enzyme levels. HO-1 is an inducible isoform of HO, and is known to be induced by substrate heme most effectively. Since HO-1 is induced also by various stresses [1], its induction constitutes an adaptive response against cellular stress [2]. Recently, we reported that up-regulation of HO-1 prevented barrier dysfunction in intestinal bleeding model [3], suggesting the novel cytoprotective function of HO-1. HO-1 is also known as HSP 32 [4]. Its 5'-regulatory region contains several potential heat shock elements, however, heat-induced expression of HO-1

differ in animal species [4-6]. HO-1 is not equally expressed in different cell types. For example, HO-1 immunoreactivity was detected in Kupffer cells, but not in parenchymal cells of normal rat liver [7, 8]. We reported that positive staining for HO-1 protein was observed not only in the Kupffer cells but also in the hepatocyte, especially around the central vein, in rat liver caused halothane-induced hepatotoxicity [9].

Among Hsps, Hsp70 is a conserved family of chaperones that facilitate folding and assembly of nascent proteins, and refolding of stress-damaged proteins [10]. The general role of Hsp70 as molecular chaperones is now central to many areas of active research including cancer and infectious disease [11]. We have previously reported that up-regulation of Hsp70 by pretreatment with heat stress promoted recovery from the ethanol-induced barrier dysfunction in intestinal epithelial cells [12]. Hsp70 might represent other functions than chaperone.

Thus, Hsps show diverse functions in living organisms, suggesting that they show different intracellular localization in order to provide cytoprotection against the stress loading. The major goal of the present study was to investigate the intracellular localization and expression changes of HO-1 and Hsp70 in H-4-II-E cells derived from rat hepatoma, after typical stress loading.

Material and Methods

Cell culture and treatments. Rat hepatoma H-4-II-E cell line was obtained from National Institute of Biomedical Innovation JCRB cell bank, Riken BioResource Center, Japan. Cells were seeded on a cover slip in a 12-well plate and maintained at 37°C in a culture medium composed of Dulbecco's modified Eagle medium with 50 U/ml penicillin, 50 U/ml streptomycin, and supplemented with heat-inactivated 10% FBS. Heme arginate (HA), a water-soluble and stable reaction product of hemin and L-arginate [13], was used for heme treatment at a final concentration of 100 μM for 6 hr. For heat shock loading (HS), cells were treated at 42°C for 1 hr. Cells were harvested immediately after HS, or 6 hr after recovering at 37°C.

Immunostaining. Intracellular localization of Hsps was assessed using fluorescent labeling techniques. H-4-II-E cells were fixed with 3.7% paraformaldehyde in PBS for 20 min, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 3% BSA in PBS. Fixed cells were incubated with rabbit polyclonal anti-heme oxygenase-1 (ADI-SPA-895), followed by incubation with anti-rabbit IgG Alexa568-conjugate (Molecular Probes, A-11011) as the secondary antibody. Immunostaining with Hsp70 was performed by using mouse monoclonal anti-Hsp70 W27 clone (Santa Cruz, sc-24) as the primary antibody and goat polyclonal anti-mouse IgG Alexa568-conjugate (Molecular Probes, A-11004) as the secondary antibody. Stained cells were mounted with DAPI fluoromount G (SouthernBiotech, 0100-20). Fluorescence was analyzed using a confocal laser scanning microscope (Olympus Fluoview FV 1000) and images were processed on Image J.

Results and Discussion

Since free heme is the strongest inducer of HO-1, we examined the intracellular localization of HO-1 induced by HA treatment. Exposure to 100 μM HA for 6 hr resulted in marked induction of HO-1 specifically in cytosol (Fig. 1). Images with higher magnification illustrated preferential localization of HO-1 in endoplasmic reticulum (ER) (Fig. 1). The induction level of HO-1 is almost the same between cells. Recently, we reported that HA treatment induced the expression of HO-1 more than 10-fold in H-4-II-E cells, measured by Western blot analysis [14]. In this study, we showed that HA treated cells up-regulated HO-1 expression homogeneously.

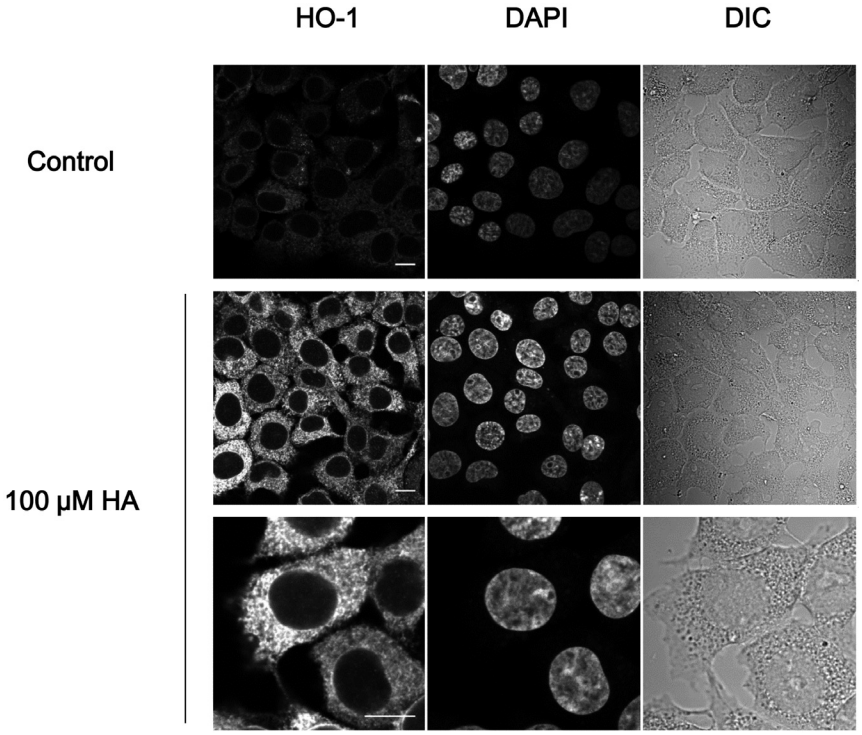

Figure 1. *Homogeneous upregulation of HO-1 by hemin treatment.*
Subconfluent H-4-II-E cells were cultured in the presence of 100 μM HA for 6 hr. HO-1 expression was accessed by immunostaining using anti-HO-1 antibody (lane HO-1). Nuclear staining was performed using DAPI (lane DAPI). Differential interference contrast images were simultaneously taken (lane DIC). A typical result taken from 3 independent experiments was shown. Bar repesents 10 μm.

While heme is required as the prosthetic group for hemoproteins that are necessary for cellular viability [15], an excess amount of free heme is deleterious, since it acts as a potent pro-oxidant, leading to the generation of oxygen radicals [16, 17]. To cope with this problem, cells equipped heme degradation system, the leading part of which is catalyzed by HO. HO enzymes have been described as ER-associated proteins, since their initial characterization in 1969 [18]. However, when HO-1 was overexpressed, inducible HO activity appeared in plasma

membrane, cytosol, and isolated caveolae [19]. On the contrary, our results showed that HO-1 induced by HA treatment mainly localized in ER in H-4- II-E cells (Fig. 1). It is noteworthy that HO form an equimolar complex with NADPH-cytochrome P450 reductase, which localize in the ER [20]. Since strong induction of HO-1 by HA treatment was aimed to degrade the toxic heme, it was suggested that heme cleavage reaction proceeds in a very efficient manner within the binary complex located in the ER [21].

We next investigated the intracellular localization of Hsp70, which was known as the major heat shock and stress-induced protein. Hsp70 distributed mainly in cytosol in untreated cells (Fig. 2). Immediately after HS, Hsp70 moved into nucleus in all cells, so far examined (Fig. 2). Intracellular localization of Hsp70 remarkably changed 6 hr after HS. Newly synthesized Hsp70 localized mainly in cytosol (Fig. 2). Recently, we reported that the expression of Hsp70 increased approximately 20-fold in H-4-II-E cells 6 hr after HS, measured by Western blot analysis [14]. In this study, we observed that the induction level of Hsp70 varied between cells (Fig. 2), in contrast to the homogeneous HO-1 expression induced by HA treatment (Fig. 1).

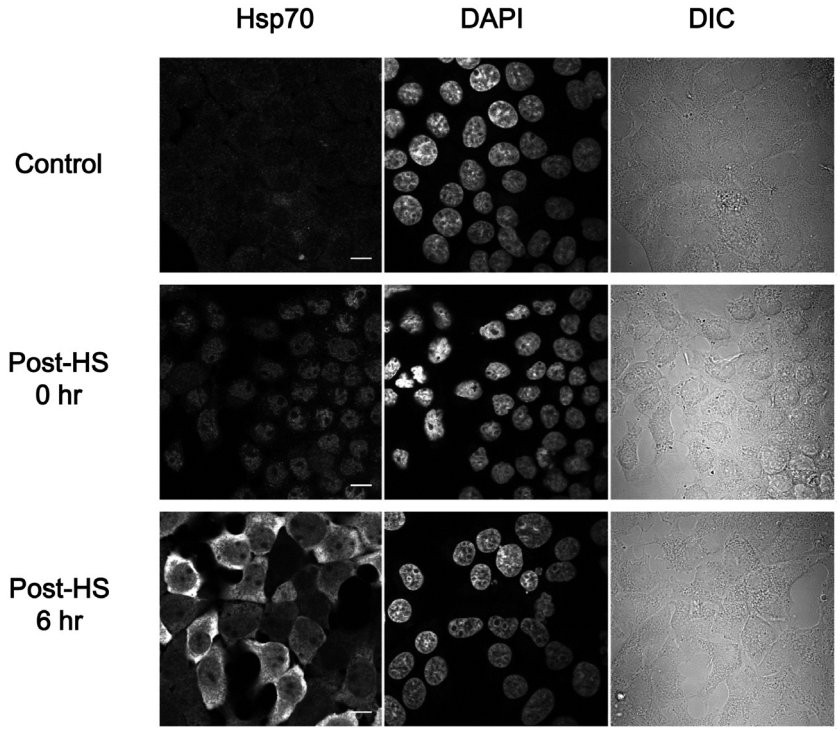

Figure 2. *Changes in intracellular localization of Hsp70 after HS.*
Subconfluent H-4-II-E cells were treated with HS (at 42°C for 1 hr), followed by recovery incubation at 37°C for 6 hr. Expression and intracellular localization of Hsp70 were analyzed by immunostaining using anti-Hsp70 antibody (lane Hsp70). To avoid signal saturation, the image for the 6 hr after HS was obtained using lower detector sensitivity (50% intensity compared to other conditions). Nuclear staining was performed using DAPI (lane DAPI). Differential interference contrast images were simultaneously taken (lane DIC). A typical result taken from 3 independent experiments was shown. Bar repesents 10 μm.

The primary function of the family of HSPs is to serve as molecular chaperones to protect thermally damaged proteins from aggregation, unfold aggregated proteins, and refold damaged proteins or target them for efficient degradation [22]. In addition to the contribution to stress responses, Hsp70 was reported to interact with cell cycle-associated proteins [23]. They observed that maximal levels of Hsp70 mRNA were detected in S-phase at the peak of DNA synthesis in HeLa cells. Our observation of the diverse intracellular localization and induction level of Hsp70 after HS might be reflected by the different phase of cell cycle.

Thus, HO-1 and Hsp70 localize independently, affected by the role against various stresses. The expression patterns of these Hsps shown in this study suggest a complementary physiological role in stress-loading cells.

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