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ScienceDirect
Cellular Signalling 20 (2008) 347–358
CELLULAR SIGNALLING
www.elsevier.com/locate/cellsig

HSP105 interacts with GRP78 and GSK3 and promotes ER stress-induced caspase-3 activation

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Received 15 August 2007; received in revised form 27 October 2007; accepted 29 October 2007
Available online 17 November 2007

Abstract

Stress of the endoplasmic reticulum (ER stress) is caused by the accumulation of misfolded proteins, which occurs in many neurodegenerative diseases. ER stress can lead to adaptive responses or apoptosis, both of which follow activation of the unfolded protein response (UPR). Heat shock proteins (HSP) support the folding and function of many proteins, and are important components of the ER stress response, but little is known about the role of one of the major large HSPs, HSP105. We identified several new partners of HSP105, including glycogen synthase kinase-3 (GSK3), a promoter of ER stress-induced apoptosis, and GRP78, a key component of the UPR. Knockdown of HSP105 did not alter UPR signaling after ER stress, but blocked caspase-3 activation after ER stress. In contrast, caspase-3 activation induced by genotoxic stress was unaffected by knockdown of HSP105, suggesting ER stress-specificity in the apoptotic action of HSP105. However, knockdown of HSP105 did not alter cell survival after ER stress, but instead diverted signaling to a caspase-3-independent cell death pathway, indicating that HSP105 is necessary for apoptotic signaling after UPR activation by ER stress. Thus, HSP105 appears to chaperone the responses to ER stress through its interactions with GRP78 and GSK3, and without HSP105 cell death following ER stress proceeds by a non-caspase-3-dependent process.

Keywords: HSP105; GRP78; UPR; Apoptosis; GSK3; ER stress

1. Introduction

The endoplasmic reticulum (ER) is a membranous organelle that provides a unique environment for the proper folding and maturation of secreted and membrane-bound proteins. Diseases, environmental factors, and pharmacological agents can perturb ER function and lead to the accumulation of misfolded proteins and ER stress [1,2]. When the ER stress is severe enough to preclude recovery from excessive accumulation of misfolded proteins, cells undergo controlled death by apoptosis [3,4]. This apoptotic method of cell death is important because it evolves internally, enabling the intracellular inactivation of potentially toxic molecules that could be released in necrotic cell death.

Eukaryotic cells have evolved an elegant but complex system to detect and react to misfolded proteins within the ER, encompassing both adaptive and apoptotic signals [1,2,5]. Thus, cells attempt to rectify the misfolded proteins while at the same time preparing for apoptosis should the ER stress be overwhelming [3,4]. A major response to ER stress involves activation of the highly conserved unfolded protein response (UPR). The UPR includes activation of the trans-ER membrane molecules PKR-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Each of these proteins is held inactive by GRP78 in the ER under basal conditions, and is activated following release when GRP78 is recruited away to misfolded proteins [6,7]. Activation of these UPR mediators leads to attenuation of translation and increased expression of a subset of stress-responsive genes [4,8].

The survival of cells following activation of the UPR depends on the severity and duration of the stress and is largely determined by the proapoptotic molecule C/EBP homologous transcription factor protein (CHOP, also called GADD153) [9,10]. The expression of CHOP, a member of the C/EBP family

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doi:10.1016/j.cellsig.2007.10.032

of transcription factors, is upregulated following ER stress by the ATF4 and ATF6 transcription factors [11]. With mild ER stress, CHOP is rapidly degraded and its expression is reduced, allowing cells to survive. However, prolonged or severe ER stress maintains high CHOP levels to promote apoptotic signaling that induces activation of the executioner caspase, caspase-3, and cell death by apoptosis [10].

In addition to CHOP, many other proteins have been identified that regulate the ultimate decision between adaptation and death following ER stress [12]. One of these is glycogen synthase kinase-3 (GSK3), a constitutively active Ser/Thr kinase composed of two similar, but not identical, isoforms, GSK3α and GSK3β [13]. We previously found [14] that GSK3 inhibitors reduced apoptosis induced by several agents that cause ER stress, including thapsigargin (an ER Ca^{++}-ATPase inhibitor) and tunicamycin (an N-linked glycosylation inhibitor), which has been confirmed in numerous studies [15–20]. Despite this considerable evidence that GSK3 promotes intrinsic apoptotic signaling following ER stress, the underlying mechanisms remain uncertain [21].

Heat shock proteins (HSPs) also have been identified as regulators of the ER stress response [22,23]. HSPs are molecular chaperones that escort and regulate the actions of many intracellular proteins. HSPs bind newly synthesized or misfolded proteins to optimize folding and function, whereas irreparably damaged proteins are directed by HSPs to sites of degradation [24]. Additionally, HSPs regulate multiple steps in the apoptotic signaling cascade [25]. Together these actions likely underlie the widely reported antiapoptotic, or survival-promoting, capabilities of HSPs. Additionally, HSPs play an important role in cellular signaling by ensuring that many key signaling proteins maintain optimally active conformations [26]. Thus, HSPs generally are considered as immensely helpful proteins that ensure proteins fold and function properly and damage is controlled.

Although HSP105 is one of the major mammalian heat shock proteins, little is known about its actions and client proteins compared with the well-known HSP70 and HSP90. HSP105 is a nucleotide exchange factor for HSP70 [27,28] and prevents aggregation of mutant androgen receptors [29] and Zn/Cu superoxide dismutase [30]. HSP105 attenuates staurosporine-induced apoptosis [31], but overexpressed HSP105α in mouse embryonal F9 cells enhanced apoptosis in response to oxidative stress [32]. However, the role of HSP105 in response to ER stress has not previously been examined.

If a cell is lethally damaged, especially during ER stress when many proteins are misfolded, we hypothesized that there must be a mechanism to ensure that mediators of the apoptotic cascade remain functional in a destructive environment. A logical candidate for this role is a molecular chaperone, a protein that serves to optimize protein and cellular functions even though in this case the outcome is cell death. In this report we present evidence that one function of HSP105 is to chaperone the process of apoptosis following ER stress. Therefore, the present study tested the effects of HSP105 on ER stress-induced apoptosis and demonstrates that HSP105 directs signaling to caspase-3 mediated apoptosis.

2. Materials and methods

2.1. Cell culture

SH-SY5Y human neuroblastoma cells where grown in RPMI 1640 medium supplemented with 10% horse serum and 5% fetal clone II (HyClone, Logan, UT). Cells were placed in serum-free media approximately 16 h prior to experimental treatments. HEK293 cells were grown in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and 15 mM HEPES (Cellgro, Herndon, VA). All media were supplemented with 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Cellgro), in humidified, 37 °C chambers with 5% CO_2. Cells were treated for the indicated times with thapsigargin, tunicamycin (Alexis, San Diego, CA), or camptothecin (Sigma Chemical Co., St. Louis, MO).

2.2. Tissue preparation

Adult, male C57BL/6 mice (Frederick Cancer Research, Frederick, MD), were decapitated, and brains were rapidly dissected in ice-cold saline. The cerebral cortex was homogenized in ice-cold lysis buffer containing 10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% NP-40, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 5 μg/ml pepstatin, 1 mM phenylmethanesulfonyl fluoride, 1 mM sodium vanadate, 50 mM sodium fluoride, and 100 nM okadaic acid. The homogenates were centrifuged at 20,800 ×*g* for 10 min to remove insoluble debris.

2.3. Immunoblotting

Cells were washed twice with phosphate-buffered saline (PBS) and were lysed with IP lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.5% NP-40, 1 mM sodium orthovanadate, 100 μM phenylmethanesulfonyl fluoride, 0.1 μM okadaic acid, 50 mM sodium fluoride, and 10 μg/ml each of leupeptin, aprotinin, and pepstatin). The lysates were sonicated and centrifuged at 20,800 ×*g* for 15 min. Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL). Samples were mixed with Laemmli sample buffer (2% SDS) and placed in a boiling water bath for 5 min. Proteins were resolved in SDS-polyacrylamide gels, transferred to nitrocellulose, and the membranes were incubated with antibodies to phospho-Ser9-GSK3β, phospho-Ser473-Akt, phospho-Thr308-Akt, phospho-Ser51-eIF2α, total eIF2α, cleaved caspase-3, cleaved poly-(ADP-ribose) polymerase (PARP), Bad (Cell Signaling, Danvers, MA), total GSK3β, active Bax (6A7), caspase-9 (BD-PharMingen/Transduction Laboratories, San Diego, CA), HSP90, HSP70, (Stressgen, Victoria, BC, Canada), CHOP/GADD153, GRP78 (Santa Cruz Biotechnology, Santa Cruz, CA), total GSK3 (Upstate Biotechnology Inc., Lake Placid, NY), HSP105 (Novocastra Laboratories, Newcastle upon Tyne, UK), MCL1 (Biovision, Mountain View, CA), GSK3α (Southern Biotech, Birmingham, AL), total Bax, caspase-3, Bcl-XL, IAPs (kind gifts from Dr. Tong Zhou, University of Alabama at Birmingham), β-actin, or V5-tag (Sigma). Immunoblots were developed using horseradish peroxidase-conjugated goat anti-mouse, goat anti-rabbit, or donkey anti-goat IgG, followed by detection with enhanced chemiluminescence.

2.4. Subcellular fractionation

Nuclear and cytosolic fractions were prepared as previously described [33] with minor modifications. Cells were washed twice with PBS and then harvested in 200 μl lysis buffer (10 mM Tris, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 0.05% NP-40, 1 mM EGTA, 1 mM sodium orthovanadate, 100 μM phenylmethanesulfonyl fluoride, 0.1 μM okadaic acid, 50 mM sodium fluoride, and 10 μg/ml each of leupeptin, aprotinin, and pepstatin). Cells were centrifuged at 2700 ×*g* for 10 min at 4 °C. The supernatant was centrifuged at 20,800 ×*g* for 15 min at 4 °C to obtain the cytosolic fraction. The pellet, containing nuclei, was washed twice in 200 μl wash buffer (5 mM HEPES, pH 7.4, 3 mM $MgCl_2$, 1 mM EGTA, 250 mM sucrose, 0.1% BSA, with protease and phosphatase inhibitors). The pellet was then resuspended in wash buffer and layered on top of 1 ml 1 M sucrose (with protease and phosphatase inhibitors), and centrifuged at 2700 ×*g* for 10 min at 4 °C. The nuclear pellet was washed in lysis buffer containing