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HSP27 phosphorylation modulates TRAIL-induced activation of Src-Akt/ERK signaling through interaction with β-arrestin2

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ABSTRACT

Heat shock protein 27 (HSP27) regulates critical cellular functions such as development, differentiation, cell growth and apoptosis. A variety of stimuli induce the phosphorylation of HSP27, which affects its cellular functions. However, most previous studies focused on the role of HSP27 protein itself in apoptosis, the particular role of its phosphorylation state in signaling transduction remains largely unclear. In the present study, we reported that HSP27 phosphorylation modulated TRAIL-triggered pro-survival signaling transduction. In HeLa cells, suppression of HSP27 phosphorylation by specific inhibitor KRIBB3 or MAPKAPK2 (MK2) knockdown and by overexpression of non-phosphorylatable HSP27(3A) mutant demonstrated that hindered HSP27 phosphorylation enhanced the TRAIL-induced apoptosis. In addition, reduced HSP27 phosphorylation by KRIBB3 treatment or MK2 knockdown attenuated the TRAIL-induced activation of Akt and ERK survival signaling through suppressing the phosphorylation of Src. By overexpression of HSP27(15A) or HSP27(78/82A) phosphorylation mutant, we further showed that phosphorylation of HSP27 at serine 78/82 residues was essential to TRAIL-triggered Src-Akt/ERK signaling transduction. Co-immunoprecipitation and confocal microscopy showed that HSP27 interacted with Src and scaffolding protein β-arrestin2 in response of TRAIL stimulation and suppression of HSP27 phosphorylation apparently disrupted the TRAIL-induced interaction of HSP27 and Src or interaction of HSP27 and β-arrestin2. We further demonstrated that β-arrestin2 mediated HSP27 action on TRAIL-induced Src activation, which was achieved by recruiting signaling complex of HSP27/β-arrestin2/Src in response to TRAIL. Taken together, our study revealed that HSP27 phosphorylation modulates TRAIL-triggered activation of Src-Akt/ERK pro-survival signaling via interacting with β-arrestin2 in HeLa cells.

1. Introduction

Heat shock proteins (HSPs) are a class of molecular chaperones whose expression is increased when cells are exposed to elevated temperature or other stresses. The HSPs subfamilies HSP90, HSP70, and HSP27 have been implicated for having an antiapoptotic role in response to various proapoptotic stimuli [1]. In addition, HSPs also interact with several intracellular signaling molecules providing stressed cells with the abilities to know whether to grow, divide, differentiate, or die [2]. Small HSPs such as HSP27 (or HSPB1) directly or indirectly participates in the regulation of apoptosis, protects the cell against oxidative stress, and are involved in the regulation of the cytoskeleton [3]. It has been demonstrated that regulation of HSP27 function depends in large part upon its phosphorylation state [4–6]. Typically, HSP27 exists in high-molecular-weight oligomers coupled to chaperone properties, but in response to cellular stimuli, such as oxidative stress, HSP27 is phosphorylated at several distinct serine residues (Ser15, Ser78, and Ser82) contributing to cytoprotective functions. The cellular function of HSP27 therefore shifts correspondingly to its phosphorylation state. Studies using knockout cells, siRNA-mediated depletion of target proteins, dominant negative mutants, and specific p38 MAPK inhibitors have led to the general accepted concept that HSP27 is phosphorylated through the p38 MAPK/MAPKAPK2 (MK2) module [7–10]

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was cloned and characterized as a potent anticancer agent in 1995 [11]. The last decade of research on TRAIL has revealed that this cytokine is truly an interesting molecule with a multitude of functions in both cancer and immunity [12]. TRAIL induces apoptosis in a variety of cancer cell lines while displaying minimal or no toxicity on normal cells [13]. Intriguingly, TRAIL signaling does not only lead to the activation of effector caspases and subsequent initiation of apoptosis, but can also induce non-apoptotic pathways, which includes the activation of NF-κB, PKB/Akt and MAPKs [14]. In TRAIL-induced survival signaling networks, p38/HSP27 phosphorylation is responsible for the catalytic activity of Akt and HSP27 modulates cell survival by its interactions with various

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binding partners, depending on the level of phosphorylated HSP27 [15,16]. However, the significance of HSP27 phosphorylation in TRAIL-induced survival pathways needs to be further elucidated.

The scaffolding proteins β-arrestins have been traditionally associated with the termination of G protein-coupled receptor signaling and with receptor desensitization [17,18]. Recently, growing evidence shows that β-arrestins also function to activate signaling cascades independently of G protein activation by serving as multiprotein scaffolds. The β-arrestin-scaffolded complexes can determine the subcellular location and specificity, promoting phosphorylation of diverse cytosolic substrates and thereby having different physiological consequences [19]. β-Arrestin regulation has been demonstrated for an ever increasing number of signaling molecules, including the mitogen activated protein kinases ERK, JNK, and p38 as well as PI3 kinase, Akt, and Src (reviewed in [19–21]). It was reported that β-arrestins recruit c-Src and Akt into a β-arrestin-scaffolded complex leading to full activation of Akt and the second signaling transduction in response to ghrelin in HEK293 and preadipocyte cells [21]. Kim et al. recently showed that p38/HSP27 phosphorylation is highly correlated with Akt activation via indirect binding to Src upon TRAIL-stimulation [16,22], however, little information is known concerning the relationship between HSP27 phosphorylation and β-arrestins in mediating Src-Akt signaling.

In this study, we investigated the role of HSP27 phosphorylation in protecting HeLa cells from TRAIL toxicity and the mechanism by which HSP27 modulates the activation of Src-Akt/ERK survival signaling upon TRAIL stimulation. We observed that suppression of HSP27 phosphorylation potentiated TRAIL-induced apoptosis and attenuated TRAIL-triggered activation of Akt and ERK survival pathways by suppressing the phosphorylation of Src. In addition, we found that HSP27 interacted with Src and scaffolding protein β-arrestin2 in response to TRAIL and suppression of HSP27 phosphorylation apparently disrupted the TRAIL-induced interaction of HSP27 and Src or interaction of HSP27 and β-arrestin2. Since physical binding was observed between β-arrestin2 and Src, we thus speculated that β-arrestin2 could recruit the formation of complex of phosphorylated HSP27/β-arrestin2/Src in response to TRAIL, resulting in activation of survival signaling. These findings highlight a novel role of HSP27 phosphorylation required in β-arrestin2-scaffolded signaling transduction.

2. Materials and methods

2.1. Antibodies and reagents

Polyclonal rabbit antibodies against PARP, procaspase-3, cleaved-caspase-8, MAPKAPK2, Src, phospho-Src (Tyr416), Akt, phospho-Akt (Ser 473), p42/p44 MAPK, phospho-p42/44 MAPK (Thr202/Tyr204) and monoclonal rabbit antibody against β-arrestin1 or β-arrestin2 were obtained from Cell Signaling Technology (Beverly, MA). Polyclonal rabbit antibody against HSP27 and monoclonal mouse antibody against HSP27 were gained from Stressgen (Victoria, BC, Canada). Phospho-HSP27 (Ser15, 78 and 82) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). Polyclonal rabbit antibody against GAPDH was from Bioworld Technology (Minneapolis, MN). Recombinant human TRAIL was the product of Peprotech (Rocky Hills, NJ). Mouse monoclonal antibody against FLAG-tag and KRIBB3 (5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl) isoxazole) were purchased from Sigma-Aldrich (St. Louis, MO). Mouse monoclonal antibody against Myc-tag was purchased from Roche Applied Science (Indianapolis, IN). PP2 and PP3 were purchased from BioVision (California, CA).

2.2. DNA constructs

pcDNA3.0-FLAG-HSP27 (WT) and HSP27 mutants including pFLAG-HSP27-3A (in which serine 15/78/82 were mutated to alanine, nonphosphorylatable), pFLAG-HSP27-3D (in which serines 15/78/82 were mutated to aspartate, phosphomimetic), pcDNA3.0-FLAG-HSP27-S15A (in which serine 15 was mutated to alanine) and pcDNA3.0-FLAG-HSP27-S78/82A (in which serine 78 and serine 82 were both mutated to alanine) were constructed by using standard techniques. pBS-U6-β-Arrestin 1/2 were kindly provided by Dr. Gang Pei (Chinese Academy of Sciences, Shanghai, PR China). All of the constructs were verified by DNA sequencing.

2.3. Cell culture and transfection

Human cervical carcinoma HeLa cells obtained from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, People's Republic of China), were cultured in Dulbecco's modified Eagle's medium (Wisent, Montreal, Quebec, Canada) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37 °C with 5% CO_2. Transient transfection was performed with X-tremeGENE 9 DNA Transfection Reagent (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. In all cases, the total amount of DNA was normalized by empty control plasmids.

2.4. Co-immunoprecipitation and immunoblot analysis

Cells were lysed on ice in the lysis buffer containing 20 mM Tris (pH7.5), 135 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM dithiothreitol (DTT), 25 mM β-glycerophoshate, 2 mM sodium pyrophosphate, 10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, 10 mM NaF and 1 mM phenyldulfonyl flouoride (PMSF) supplemented with complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). Lysates were centrifuged (15,000 ×*g*) at 4 °C for 15 min. Proteins (0.5 mg) were immunoprecipitated with indicated antibodies (0.5 μg) separately. The precleared Protein A/G PLUS-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated with immunocomplexes for another 2 h and washed four times with the lysis buffer. The immunoprecipitates were subjected to SDS-PAGE followed by transferring onto nitrocellulose membranes (Whatman, GE Healthcare, NJ). The antibody-antigen complexes were visualized by the LI-COR Odyssey Infrared Imaging System according to the manufacturer's instruction using IRDye800 flurophore-conjugated antibody (LI-COR Biosciences, Lincoln, NE). Quantification was directly performed on the blot using the LI-COR Odyssey Analysis software. Aliquots of whole cell lysates were subjected to immunoblotting to confirm appropriate expression of proteins.

2.5. RNA interference

Small hairpin RNA (shRNA) constructs against HSP27 mediated by pRS shRNA vector (catalog number TR320383) and pRS negative control (catalog number TR20003) were purchased from Origene (Rockville, MD). shRNA constructs against MAPKAPK2 (catalog number 62-143) and pKD-NegCon-v1 (catalog number 62-002) were purchased from Upstate Biotechnology (Lake Placid, NY). pBS-U6-β-Arrestin 1/2 were kindly provided by Dr. Gang Pei (Chinese Academy of Sciences, Shanghai, PR China). HeLa cells were transfected with shRNA or negative control vector using X-tremeGENE 9 DNA Transfection Reagent (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Interference efficiency was confirmed by immunoblot analysis after 72 h transfection using HSP27, MK2, β-arrestin1 or β-arrestin2 antibody, respectively.

2.6. AnnexinV-FITC/PI assay

The apoptosis assay was performed with a two color analysis of FITC-labeled AnnexinV binding and PI uptake using the Annexin V-Fluos staining kit (BD Pharmingen, San Diego, CA, USA). After the indicated treatment, 10^5 cells were resuspended in 50 μl phosphate-buffered