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Jagadish C. Ghosh  
*University of Massachusetts Medical School*

Takehiko Dohi  
*University of Massachusetts Medical School*

Byoung Kang  
*University of Massachusetts Medical School*

*See next page for additional authors*

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Hsp60 Regulation of Tumor Cell Apoptosis*

Jagadish C. Ghosh, Takehiko Dohi, Byoung Heon Kang, and Dario C. Altieri

From the Department of Cancer Biology and the Cancer Center, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Molecular chaperones may promote cell survival, but how this process is regulated, especially in cancer, is not well understood. Using high throughput proteomics screening, we identified the cell cycle regulator and apoptosis inhibitor survivin as a novel protein associated with the molecular chaperone Hsp60. Acute ablation of Hsp60 by small interfering RNA destabilizes the mitochondrial pool of survivin, induces mitochondrial dysfunction, and activates caspase-dependent apoptosis. This response involves disruption of an Hsp60-p53 complex, which results in p53 stabilization, increased expression of pro-apoptotic Bax, and Bax-dependent apoptosis. In vivo, Hsp60 is abundantly expressed in primary human tumors, as compared with matched normal tissues, and small interfering RNA ablation of Hsp60 in normal cells is well tolerated and does not cause apoptosis. Therefore, Hsp60 orchestrates a broad cell survival program centered on stabilization of mitochondrial survivin and restraining of p53 function, and this process is selectively exploited in cancer. Hsp60 inhibitors may function as attractive anticancer agents by differentially inducing apoptosis in tumor cells.

In particular, Hsp60, together with its associated chaperonin, Hsp10, has been recognized as an evolutionarily conserved stress response chaperone (10), largely but not exclusively compartmentalized in mitochondria (11) and with critical roles in organelle biogenesis and folding/refolding of imported preproteins (12). However, whether Hsp60 also contributes to cell survival is controversial, with data suggesting a pro-apoptotic function via enhanced caspase activation (13, 14) or, conversely, an anti-apoptotic mechanism involving sequestration of Bax-containing complexes (15). A role of Hsp60 in cancer is equally uncertain, as up-regulation (16, 17) or down-regulation (18, 19) of this chaperone has been reported in various tumor series correlating with disease outcome.

There is now accumulating evidence that molecular chaperones play a key role in regulating the function of survivin (20), one of the most “cancer-specific” genes (21) involved in protection from apoptosis and control of mitosis in transformed cells. Accordingly, binding of survivin to Hsp90 (22) or the immunophilin aryl hydrocarbon receptor-interacting protein (AIP) (23) maintains survivin stability against proteasome-dependent destruction (22, 23) and preserves an anti-apoptotic threshold in tumor cells (24).

In this study, we used high throughput proteomics screening to identify novel binding partners of survivin that potentially contribute to its tumorigenic role. We found that Hsp60 (12) associates with survivin, and this recognition contributes to a broad anti-apoptotic program differentially exploited in tumors in vivo.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—Breast adenocarcinoma cells MCF-7 and MDA-MB-231, colon adenocarcinoma cells HCT116, normal intestinal epithelial cells Fhs 741NT, and primary WS-1 or HFF normal fibroblasts were obtained from the American Type Culture Collection (Manassas, VA) and maintained in culture as recommended by the supplier. Wild-type (WT), p53−/−, and Bax−/− HCT116 cells were kindly provided by Dr. B. Vogelstein (Johns Hopkins University, Baltimore, MD). To generate a cell line stably expressing survivin, MCF-7 cells were transfected with survivin cDNA by Lipofectamine 2000 (Invitrogen) and selected in a medium containing 1 mg/ml G418 (Invitrogen), and the colonies were picked after 2–3 weeks. Transfected clones were expanded and confirmed for overexpression of survivin (3–4-fold over endogenous levels) by Western blotting. Antibodies against Hsp60 (BD Transduction Laboratories), survivin (NOVUS Biologicals), p53 (Oncogene Research Products and Santa Cruz Biotechnology), p21 (Oncogene), Bax (Oncogene), Mdm-2 (Santa

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1 To whom correspondence should be addressed: Dept. of Cancer Biology, LRB428, University of Massachusetts Medical School, 364 Plantation St., Worcester, MA 01605. Tel.: 508-856-5775; Fax: 508-856-5791; E-mail: dario.altieri@umassmed.edu.

2 The abbreviations used are: Hsp, heat shock protein; siRNA, small interfering RNA; WT, wild type; CCCP, carbonyl cyanide p-chlorophenylhydrazone; GST, glutathione S-transferase; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.
transfection experiments, cells were loaded twice with control or Hsp60-directed siRNA at 48-h intervals between each transfection.

**Mitochondrial Import Assay**—The import of recombinant survivin into purified mitochondria was carried out as described (3). Briefly, aliquots of 35S-labeled in vitro transcribed and translated survivin (TNT system, Promega) were diluted with 1 volume of MC buffer containing sucrose (500 mM sucrose, 80 mM potassium acetate, 20 mM HEPES-KOH, pH 7.5, 5 mM magnesium acetate) and mixed in a total volume of 50 μl with purified mouse brain mitochondria (30 μg) for 1 h at 30°C in the presence or absence of increasing concentrations of unlabeled survivin. At the end of the incubation reaction, mitochondria were resolated by centrifugation at 6,000 × g for 10 min, and the differential protein accumulation in mitochondria was determined by autoradiography.

**Analysis of Apoptosis**—Transfected cells were harvested after 48 h and stained with a fluorescein-conjugated caspase inhibitor (carboxyfluorescein (FAM)-DEVD-fluoromethyl ketone) and propidium iodide (CaspaTag, Intergen) for simultaneous detection of DEVDase (caspase) activity and loss of plasma membrane integrity by multiparametric flow cytometry as described (26). Data were analyzed using FlowJo software. To quantify mitochondrial membrane potential, transfected cells were loaded with the fluorescent dye JC-1 (10 μM) (Molecular Probes) and analyzed for changes in red-green (FL-2/FL-1) fluorescence ratio by flow cytometry. Cells treated with CCCP (50 μM, for 5 min) served as a positive control.

**Subcellular Fractionation**—Mitochondrial and cytosolic fractions were extracted from tumor cells (6–7 × 106) as described (27).

**Pulldown and Immunoprecipitation**—Purified GST fusion proteins (5 μg) were mixed with mitochondrial extracts from MCF-7 or HCT116 cells, and bound proteins were analyzed by Western blotting as described (22). Alternatively, 35S-labeled survivin or 35S-labeled Hsp60 was mixed with GST, GST-Hsp60, or GST-survivin, and bound proteins were detected by autoradiography. For immunoprecipitation, cytosolic or mitochondrial extracts from HCT116 or MCF-7 cells were incubated with IgG or an antibody to survivin or p53, and the immune complexes were precipitated by the addition of protein A-Sepharose beads (Amersham Biosciences) in 50 mM Tris, pH 7.5, 0.1% deoxycholate, 1% Nonidet P-40, 0.1% SDS, 50 mM NaCl, 1 mM protease inhibitors (Roche Applied Science), and 1 mM Na3VO4. After washing, pellets or supernatants were separ-
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rated by SDS-gel electrophoresis and analyzed by Western blotting.

Gene Profiling—Relative levels of Hsp60 transcripts in various patient cohorts with diagnoses of adenocarcinoma of colon (28), lung (29), prostate (30), glioblastoma multiformis (31), or their matched normal tissues were analyzed using Oncomine (32) and normalized as follows. Log, was transformed, the array median was set to 0, the array standard deviation was set to 1, the mean was centered per study, and these were plotted as normalized expression units.

Immunohistochemistry—Primary tissue specimens of breast, lung, and colon adenocarcinoma or matched normal tissues were obtained anonymously from the University of Massachusetts Cancer Center tissue bank. Tissue sections were processed for immunohistochemistry using IgG or an antibody to Hsp60 (1:1000) as described (27).

RESULTS

Hsp60 Is a Novel Survivin-associated Molecule—To identify novel proteins that bind survivin, we used high throughput proteomics screening with fractionation of MCF-7 cell extracts over GST-survivin and identification of bound proteins by two-dimensional gel electrophoresis and mass spectrometry (Fig. 1A, left panel). A protein spot with an apparent molecular mass of 69.6 kDa and a pI of 5.05 was detected in eluates of GST-survivin, but not GST (right panel), and identified as Hsp60 by mass spectrometry of the following matched peptide sequences: GANPVEIRR, GIIDPTKVVR, TVIIEQSWGSPK, GYISPY.

Hsp60 Regulation of Survivin Stability—Single or double transfection of MCF-7 cells with non-targeted siRNA had no effect on Hsp60 or survivin levels (Fig. 2A). Conversely, transfection of tumor cells with a single or pool siRNA oligonucleotide to Hsp60 resulted in significant reduction of Hsp60 expression (Fig. 2A). This was associated with concomitant loss of survivin levels, in a reaction that was maximal in MCF-7 cells doubly transfected with siRNA to Hsp60 (Fig. 2A). In control experiments, Hsp60-directed siRNA had no effect on the expression of an unrelated chaperone, Hsp27 (Fig. 2B), whereas it reduced Hsp6 levels in both its mitochondrial and non-mitochondrial (11) compartments (Fig. 2C). All subsequent experiments of Hsp60 knockdown were carried out with a single transfection of siRNA oligonucleotide. To determine the basis of survivin decrease after Hsp60 knockdown, we performed cycloheximide block experiments. Hsp60 knockdown under these conditions resulted in rapid destabilization and accelerated degradation of survivin protein, as compared with control cultures transfected with non-targeted siRNA (Fig. 2D).

Hsp60 Targeting Induces Mitochondrial Apoptosis—Transfection of MCF-7 or WT HCT116 cells with control siRNA did not result in DEVDase, i.e. caspase, activity or a significant decrease in cell viability measured by multiparametric flow cytometry, as compared with non-transfected cultures (Fig. 3A). In contrast, acute knockdown of Hsp60 in tumor cell lines caused loss of plasma membrane integrity and increased caspase activity (Fig. 3A). In addition, Hsp60 knockdown in tumor cells resulted in homogenous loss of mitochondrial membrane potential (Fig. 3C), discharge of mitochondrial cytochrome c in the cytosol (Fig. 3D), and proteolytic processing of effector caspase-3 to active fragments of 17 and 19 kDa (Fig. 3E). In control experiments, non-targeted siRNA had no effect on mitochondrial integrity or procaspase-3 cleavage in MCF-7 cells (Fig. 3, C–E). To determine whether loss of survivin after Hsp60 knockdown contributed to this cell death response, we generated MCF-7 clones stably transfected to express survivin (Fig. 3B, MCF-7.SV). Hsp60 knockdown in these cells did not induce caspase activity or loss of plasma membrane integrity, as compared with transfected parental MCF-7 cells (Fig. 3A, bottom panel).

Hsp60 Regulation of Mitochondrial Survivin—A pool of survivin localized to mitochondria is specifically earmarked to...
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inhibit apoptosis, and this mechanism enhances tumor growth in vivo (33). To determine whether cell death induced by Hsp60 knockdown involved the mitochondrial pool of survivin, we first carried out mitochondrial import studies in vitro. 35S-Labeled survivin was readily imported in isolated mouse brain mitochondria in a reaction progressively out-competed by excess unlabeled survivin (Fig. 4A). Analysis of isolated subcellular fractions revealed that Hsp60 knockdown resulted in a nearly complete loss of mitochondrial survivin levels, whereas the cytosolic pool of survivin was largely unaffected (Fig. 4B). In these experiments, acute ablation of Hsp60 by siRNA was also associated with increased expression of p53 in both cytosol and mitochondrial extracts, as compared with non-targeted siRNA (Fig. 4B).

Because some Hsp molecules have been shown to regulate p53 (34, 35), next we further investigated potential modulation of p53 expression by Hsp60. siRNA silencing of Hsp60 in WT HCT116 cells resulted in increased expression of p53 and in parallel up-regulation of p53-responsive genes, Bax, and, to a lesser extent, p21 (Fig. 4C). In contrast, no changes in Bax expression were observed in p53−/− HCT116 cells transfected with control or Hsp60-directed siRNA (Fig. 4C).

Hsp60 Knockdown Induces p53-dependent Apoptosis—At variance with cells carrying WT p53, transfection of p53−/− HCT116 or p53 mutant MDA-MB-231 cells with Hsp60-directed siRNA did not result in caspase-dependent cell death or loss of plasma membrane integrity, as compared with untreated cultures or cells transfected with non-targeted siRNA (Fig. 5A). To map the potential requirement of p53 for cell death induced by Hsp60 knockdown, we next analyzed HCT116 cells deficient in Bax, a p53 target gene up-regulated by Hsp60 siRNA treatment. In these experiments, transfection of Bax−/− HCT116 cells with Hsp60-directed siRNA had no effect on caspase activity or plasma membrane integrity (Fig. 5B) and did not result in loss of mitochondrial membrane potential (Fig. 5C). A control, non-targeted siRNA did not affect cell viability or mitochondrial integrity of Bax−/− HCT116 cells (Fig. 5B and C).

Identification of Hsp60-p53 Complexes—We next asked whether Hsp60 physically associated with p53, thus potentially restraining its function. In pulldown experiments, GST-Hsp60, but not GST alone, associated with p53 in extracts isolated from WT HCT116 cells (Fig. 6A). In addition, p53 that was immuno-

FIGURE 3. Hsp60 knockdown induces mitochondrial apoptosis. A, caspase activity. WT HCT116, MCF-7, or MCF-7 cells stably expressing survivin (MCF-7 SVV) were transfected with the indicated siRNA and analyzed for DEVDase (caspase) activity (green fluorescence) and propidium iodide (red fluorescence) staining by multiparametric flow cytometry. None, non-transfected cells. For A and C, the percentage of cells in each quadrant is indicated. B, characterization of survivin-expressing MCF-7 cells. Parental MCF-7 cells or MCF-7 cells stably transfected with a survivin cDNA were analyzed by Western blotting. Cox-IV and Hsp60 were the mitochondrial and cytosolic markers, respectively. Ctrl, control. C, cytochrome c (Cyto c) release. Tumor cell types were transfected with the indicated siRNA and analyzed by Western blotting. Ctrl, control. E, active caspase-3 (Casp.3) generation. MCF-7 cells transfected with the indicated siRNA were analyzed by Western blotting.

FIGURE 4. Regulation of mitochondrial survivin and p53 expression by Hsp60. A, mitochondrial import of survivin. 35S-Labeled survivin was incubated with mouse brain mitochondria in the presence or absence of the indicated concentrations of unlabeled survivin, and radioactivity associated with mitochondria was determined by autoradiography. Inp, input. B, regulation of mitochondrial survivin stability by Hsp60. MCF-7 cells transfected with the indicated siRNA were fractionated in cytosol or mitochondrial (Mito) extracts and analyzed by Western blotting. Cox-IV and β-actin were the mitochondrial and cytosolic markers, respectively. Ctrl, control. C, p53 stabilization by Hsp60 knockdown. WT or p53−/− HCT116 cells were transfected with the indicated siRNA and analyzed by Western blotting.
precipitated from cytosol or mitochondrial extracts of WT HCT116 cells contained co-associated Hsp60 (Fig. 6B). In contrast, control immune complexes that were precipitated with IgG did not contain Hsp60 (Fig. 6B). We next looked at potential changes in the expression of the p53 negative regulator, Mdm-2, in transfected cells. Transfection of MCF-7 or WT HCT116 cells with Hsp60-directed siRNA did not significantly affect Mdm-2 levels, as compared with control cultures transfected with non-targeted siRNA (Fig. 6C).

Selective Hsp60 Cytoprotection in Tumors—To determine whether Hsp60 cytoprotection was preferentially exploited in cancer, we next examined its expression and function in normal versus tumor cell types. Hsp60 was abundantly present in mitochondrial and extramitochondrial (11), i.e. cytosolic, fractions of MCF-7 and HCT116 cells (Fig. 7A, left panel). In contrast, primary WS-1 and HFF human fibroblasts exhibited considerably reduced levels of Hsp60 in both subcellular compartments (right panel). By immunohistochemistry, Hsp60 was undetectable or expressed at very low levels in normal epithelium of breast, colon, and lung in vivo (Fig. 7B). In contrast, Hsp60 was abundantly expressed in the tumor cell population of adenocarcinomas of the breast, colon, and lung (Fig. 7B). In control experiments, IgG did not stain normal or tumor epithelia (data not shown). Consistent with these data, meta-analysis of published microarray data sets revealed that Hsp60 expression was considerably up-regulated in patient samples of adenocarcinoma of colon, lung, prostate, and glioblastoma multiformis, as compared with matched normal tissues (Fig. 7C). To determine whether Hsp60 cytoprotection was selectively operative in tumor cells, we next targeted Hsp60 expression in normal cell types and analyzed cell viability. Transfection of 74INT normal human epithelial cells or WS-1 primary human fibroblasts with Hsp60-directed siRNA suppressed Hsp60 expression, whereas a non-targeted siRNA was without effect (data not shown). At variance with the results obtained with tumor cell lines, acute siRNA ablation of Hsp60 in normal cells did not result in loss of cell viability or increased caspase activity, as compared with control cultures transfected with non-targeted siRNA (Fig. 7D).

**DISCUSSION**

In this study we have shown that the molecular chaperone Hsp60 (12) is prominently up-regulated in human cancers in vivo and orchestrates a cytoprotective pathway centered on stabilization of survivin levels and restraint of p53 function (Fig. 8). Conversely, acute ablation of Hsp60 results in loss of the mitochondrial pool of survivin, which is specifically earmarked to inhibit apoptosis (33), parallel increased expression of p53, and activation of p53-dependent apoptosis in tumor cells (Fig. 8). This dual cytoprotective mechanism of Hsp60 is selectively exploited in tumors in vivo, where Hsp60 is differentially up-regulated, as compared with normal tissues, and loss of Hsp60 in normal cells is not associated with mitochondrial dysfunction or cell death.

Although survivin is recognized as a pivotal cancer gene with dual roles in cell division and inhibition of apoptosis (36, 37), the molecular requirements of how survivin contributes to tumorigenesis have not been completely elucidated. However, a critical requirement of this process is the presence of a pool of survivin compartmentalized in mitochondria, preferentially in tumor cells, and released in the cytosol in response to cell death stimuli (27). There is now evidence that mitochondrial survivin provides for a pool of the molecule specifically earmarked to inhibit apoptosis (37), thus directly accelerating tumor growth in vivo (27), and that this pathway is regulated by compartmentalized phosphorylation and differential binding to the anti-apoptotic cofactor XIAP (X chromosome-linked inhibitor of apoptosis) (33). Despite the lack of a cleavable, amino-terminal mitochondrial import sequence, we have shown here that survivin is actively imported in isolated mitochondria. This dual cytoprotective mechanism of Hsp60 is selectively exploited in tumors in vivo, where Hsp60 is differentially up-regulated, as compared with normal tissues, and loss of Hsp60 in normal cells is not associated with mitochondrial dysfunction or cell death.

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Reminiscent of the model presented here, mitochondrial Hsp70, also called mortalin, has been shown to bind and sequester p53 in the cytosol (34, 41), thus preventing its nuclear import, and in centrosomes (42), where this interaction overrides a p53-dependent checkpoint of centrosomal duplication. Hsp60 regulation of p53 does not appear to involve changes in the p53 regulator Mdm-2 and thus is distinct from the role of inducible Hsp70 in antagonizing p53-dependent senescence (35). Finally, although a mitochondrial pool of p53 has been described that promotes apoptosis via modulation of Bcl-2 family proteins (43), and loss of Hsp60 resulted in increased expression of p53 in mitochondria, the data presented here implicate that de novo p53-dependent transcription, i.e. Bax induction, is required for apoptosis initiated by Hsp60 targeting.

Given their interface with multiple pathways of tumor maintenance (8) and their frequent overexpression in cancer in vivo, molecular chaperones have been vigorously pursued for novel cancer therapeutics (9). Taken together (4), the data presented here suggest that cytoprotection may be a general property of multiple molecular chaperones, including Hsp60, aimed at elevating an anti-apoptotic threshold in tumor cells in vivo. Intriguingly, this process appears to be exploited selectively in transformed cells but not in normal tissues. In addition to a sharp differential expression of these chaperones, i.e. Hsp60, in cancer as opposed to normal tissues in vivo, other functional factors may contribute to the preferential utilization of this pathway in tumor cells. These may include qualitative changes in chaperone activity, as has been demonstrated for Hsp90 ATPase function (44), or association with cancer genes differentially expressed in cancer, as is the case for functional survivin-chaperone complexes (22, 23). Although chaperone-directed cytoprotection may promote tumor cell survival and favor drug resistance, the differential expression and/or functional exploitation of this pathway in tumor cells may be desirable for broader, chaperone-directed anticancer strategies. Validating this concept, molecular or pharmacologic targeting of complexes between survivin and Hsp90 (24), mortalin and p53 (45), and Hsp60 and survivin/p53 (this work) has been consis-

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**FIGURE 7. Differential Hsp60 expression and function in tumor cells.** A, comparative analysis of normal and tumor cells. Isolated cytosolic (C) or mitochondrial (M) fractions from MCF-7 or HCT116 cells (left panel) or primary WS-1 or HFF normal human fibroblasts (right panel) were analyzed by Western blotting. B, immunohistochemistry. Primary human tissue specimens of adenocarcinoma of breast, colon, or lung (Tumor) or matched normal tissues (Normal) were stained with an antibody to Hsp60 and analyzed by immunohistochemistry. Magnification, ×200. C, gene profiling. Microarray data sets of samples (number of cases are in parentheses) of human adenocarcinoma (AdCa) of colon (28), lung (29), prostate (30), glioblastoma multiformis (31), or matched normal tissues were analyzed using Oncomine. The p values for differences in Hsp60 mRNA expression between tumor and normal samples are 1.6E-6 (colon), 1.9E-8 (lung), 7.6E-9 (prostate), and 8.7E-11 (glioblastoma multiformis). Data are shown as boxplots, where whiskers are the minimum and maximum, and boxes are the upper and lower quartiles. D, differential sensitivity of normal cells to Hsp60 knockdown. 74INT normal epithelial cells or primary WS-1 normal fibroblasts were transfected with the indicated siRNA and analyzed by DEVDase activity and propidium iodide staining by multiparametric flow cytometry. The percentage of cells in each quadrant is indicated. None, non-transfected cells.
Hsp60-mediated Cell Survival

![Diagram of Hsp60-mediated Cell Survival]


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REFERENCES