Repression of hsp90β Gene by p53 in UV Irradiation-induced Apoptosis of Jurkat Cells*

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Tumor suppressor p53 has been implicated in cell stress response and determines cell fate of either growth arrest or apoptosis. Heat shock proteins (Hsps) expressed under stress usually confer survival protection to the cell or interruption in the apoptotic pathways. Although Hsp90 can physically interact with p53, whether or not the hsp90 gene is influenced downstream of p53 in UV irradiation-induced apoptosis remains unclear. We have found that the level of p53 is elevated with the decline of Hsp90 in UV-irradiated cells and that malfunction of Hsp90, as inhibited by geldanamycin, enhances the p53-involved UV irradiation-induced apoptosis. In addition, the expression of the hsp90β gene was reduced in both UV-irradiated and wild type p53-transfected cells. These results suggest a negative correlation between the trans factor p53 and a chaperone gene hsp90β in apoptotic cells. Mutation analysis demonstrated that the p53 binding site in the first exon was dispensable for p53 regulation on the hsp90β gene. In addition, with p53 bound at the promoter of the hsp90β gene, mSin3a and p300 were differentially recruited in UV irradiation-treated or untreated Jurkat cells in vivo. The evidence of p53-repressed hsp90β gene expression in UV-irradiated cells shed light on a novel pathway of Hsp90 in the survival control of the stressed cells.

Heat shock proteins (Hsps)¹ are a large family of highly conserved proteins broadly categorized according to their size and functions. Some of the Hsps are constitutively expressed, whereas others are rapidly induced in response to cellular stress. Hsps can protect the cells from potentially fatal consequences of adverse environmental, physical, or chemical stresses to the cells. The protecting role is attainable by the chaperone functions of Hsps in prohibiting protein aggregation and promoting refolding of the denatured proteins in the stressed cells (1). Among the Hsps, the Hsp90 family is ubiquitously expressed and is one of most abundant cytoplasmic proteins. It not only participates in the protection of cell survival but also functions as a specific molecular chaperone in cell growth and differentiation (2–4). Despite the fact that Hsp90 is constitutively abundant in mammalian cells, the protein can be further induced by heat shock and to a lesser extent by mitogen in human T lymphocytes (5). Induced expression of Hsp90 in a stressed cell may strengthen cellular resistance to stress-induced apoptotic pathways (6). In the context of its functions, Hsp90 antagonists, such as geldanamycin (GA) and its derivatives, are adopted as cancer therapeutic drugs in clinical trials (6, 7).

The tumor suppressor p53 takes part in cell cycle control, DNA damage repair, and apoptosis (8, 9). However, its importance is frequently underestimated in that the p53 gene is frequently mutated in more than 50% of all human tumors. p53 acts as a nuclear transcription factor that is latent in normal cells but becomes activated by a variety of cellular stresses, DNA damage, hypoxia, etc. (10). It can transactivate a series of genes involved in cell cycle arrest and apoptosis, typically the p21/WAF1 gene (11, 12). p53 also negatively regulates a number of target genes, including Bcl-2, Bcl-x, and the survivin gene, etc. (13–15). It is thus clear that p53-dependent apoptosis is based on both the activation of proapoptotic genes and the repression of antiapoptotic genes (16).

Based on the facts that GA could disrupt the antiapoptotic activity and the stability of survivin (6) and that p53 trans repressed the expression of the survivin gene (14, 15) in cell stress response, we suggest that some direct linkage between Hsp90-survivin and p53 may exist under stress. In addition, as Hsp90 can physically interact with either the mutant (17, 18) or the wild type p53 (19, 20) in vivo, the question of whether the hsp90 gene could be downstream of p53 is of importance. These and other findings prompted us toward intensive work on the relationship between p53 and the hsp90 gene in UV irradiation-induced apoptosis.

In this paper, we provided the first evidence that wild type p53 bound to its binding site within the hsp90β gene was a prerequisite for the trans repression of p53 on the hsp90β gene in UV irradiation-induced apoptotic Jurkat cells. It also revealed a novel means of counteractions between wild type p53 and Hsp90, the repression of hsp90β gene expression to eliminate its functions in the apoptotic cells.

EXPERIMENTAL PROCEDURES

Plasmids and Antibodies—The eukaryotic expression plasmids pC53-SN3 and pC53-SCx3 are gifts from Dr. B. Vogelstein (Johns Hopkins Oncology Center, Baltimore, MD). pC53-SN3 is a plasmid of wild type human p53 cDNA, and pC53-SCx3 is a construct of mutated human p53 with Val-143 substituted by Ala. A DNA fragment spanning 1039 to +1531 of the hsp90β gene was fused to the upstream region of a chloramphenicol acetyltransferase (CAT) reporter gene in
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Cell Culture and UV Irradiation—Jurkat cells were grown in RPMI 1640 medium (Invitrogen) with 10% fetal calf serum, 0.05% l-glutamine, 0.2% NaHCO₃, 0.59% HEPES at pH 7.2, and sodium penicillin and streptomycin sulfate (100 units/ml each) in a 5% CO₂ humidified atmosphere at 37 °C. In this paper, Jurkat cells were UV-irradiated at 20 J/m² with a UV cross-linker (Bio-Rad, GS Gene Linker™, UV Chamber) and then harvested at different time points postirradiation for studying the induction of apoptosis and related gene expression. Jurkat cells were also treated with GA, a specific inhibitor for Hsp90 function, at a final concentration of 5 μM for 16 h (2, 24, 25) to explore the function of Hsp90 in the system. UV irradiation on GA-treated cells was also applied to Jurkat cells for investigating Hsp90 function in UV irradiation-induced apoptosis. GA is a gift from Dr. L. Neckers, NCI, National Institutes of Health, Rockville, MD.

DNA Transfection and Promoter Activity Assay—Electroporation was used to perform transfection of DNA into Jurkat cells in this study (Gene Pulser II, Bio-Rad). DNA extractions of reporter plasmid (hsp90β-CAT) and transfection control plasmid (pM-CAT) were mixed at the appropriate molar ratio for transfection into Jurkat cells to normalize promoter activity of the gene (22, 23). To study p53 effects, constructs pC53-SN3 or pC53-SCX3 were co-transfected with hsp90β-CAT and pM-CAT into Jurkat cells. At 48 h posttransfection, cells were separated into two groups and incubated at either 42 or 37 °C for 1 h. Total cellular RNA was extracted and used for detecting promoter activity of the hsp90β gene in a competitive RT-PCR-based system as described previously (22, 23). A pair of primers mapped to 554/573 (5′-TATTTGTTCTGTCTGGGTATCGGAAAGCAAGCCT-3′) and 698/699 (5′-CGTGTGCATCATCCTTCG-3′) were used for PCR amplification from genomic DNA. PCR products thus recovered were further treated with proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation in the presence of glycogen and sodium acetate sequentially and were resuspended in 100 μl of distilled water. 10 μl of each DNA sample thus obtained was used in PCR analysis. For negative control, 200 μl of chromatin was treated the same as that of IP except preimmune serum was used instead of specific antibody.

Western Blot Assay—Western blot assay were performed as described elsewhere (28, 29) with minor modifications. Aliquots of whole cell lysate were separated on SDS-PAGE and electrotransferred to nitrocellulose filters in a Trans-Blot cell (Bio-Rad). Filters were blocked for 1 h in blocking buffer and then incubated overnight at 4 °C using antibodies against acetylated p53, p55, PARP, or Hsp90β as required, or one by one after stripping.

Quantification of Cellular mRNA of hsp90β—RT-PCR-based mRNA quantification for hsp90β in Jurkat cells was carried out as described previously (23, 30). Briefly, an internal control RNA (icRNA) was first transcribed in vitro from pHSY3 plasmid, which contains the same 5′- and 3′-flanking sequences as hsp90β gene. An equal amount of icRNA was then mixed with each aliquot of cellular RNA, reverse transcribed, and amplified in the competitive RT-PCR system. The size of amplified fragments for hsp90β mRNA and icRNA was 337 and 625 bp, respectively. RT-PCR products of hsp90β mRNA and icRNA were separated on 1.5% agarose gel; the bands showed up with ethidium bromide and scanned with UltraScan XL or AlphaImager 2000™. The ratio of the darkness of bands in each individual lane (mRNA/icRNA) was defined as the relative expression level of hsp90β mRNA.

Detection of Apoptosis by Fluorescence-activated Cell Sorting (FACS)—Cells were immediately treated at 37 °C after UV irradiation (20 J/m²) and harvested at the indicated times, followed by washing with phosphate-buffered saline and fixing in 70% ethanol at 4 °C overnight, sequentially. Following washing with phosphate-buffered saline two times, cells were stained by propidium iodide (PI, Sigma) containing 100 μg/ml RNase A (Roche Applied Science) at 37 °C for 30 min, and apoptosis was detected by FACS using Coulter® Epic XL™.
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Fig. 1. Effects of geldanamycin on UV irradiation-induced apoptosis in Jurkat cells. The cells were pretreated with or without 5 μM GA (+GA or −GA, respectively) followed by exposure to UV irradiation (20 J/m²) and harvested at each indicated recovery time of 0, 1, 2, 4, 6, and 9 h post-UV irradiation (shown as Post-UV (h) in the figure). A, FACS analysis. The percentage of apoptotic cells observed in each FACS analysis sample was inserted in the left-hand corner of each graph as digits (%). B, Western blotting assay. PARP and its proteolytic product with relative molecular mass of 113 and 89 kDa, respectively, were separated on 8% SDS-polyacrylamide gel and blotted with antibody against PARP. The protein loaded is shown at the bottom of each individual lane with fast green staining. C, analyses for the efficiency of PARP proteolysis shown in B. Efficiency of PARP proteolysis was calculated as the ratio of the density of the lower band to the upper band in each lane scanned with a Alphalager 2200™.

RESULTS

Geldanamycin-enhanced UV Irradiation-induced Apoptosis in Jurkat Cells—UV irradiation was known to be genome-toxic to mammalian cells that induce DNA repair, cell cycle arrest, or apoptosis. Jurkat cells exposed to UV irradiation (20 J/m²) were adopted as the model system here. In addition, GA, a specific functional inhibitor of Hsp90 (2, 24, 25, 33), was applied to explore the function of Hsp90 in apoptosis. It was known that Hsp90 was capable of conferring survival signal to the stressed cells via interrupting the caspase activation pathway (34, 35). Consequently, besides the FACS assay, proteolysis of PARP, one of the earliest irreversible events in apoptosis (36), was also shown.

When the cells were exposed to UV irradiation, 34% underwent apoptosis at 9 h of recovery (Fig. 1A, top), comparable with the results of others (37, 38). Proteolysis of PARP also increased in UV-irradiated cells (Fig. 1B, top), as shown by the ratio of the densities of the lower band to the upper band in each lane. In Jurkat cells treated with GA alone, the percentage of apoptotic cells in FACS (Fig. 1A, lane 1) and the cleavage of PARP (Fig. 1B, lane 1) are slightly higher than that of the untreated control. In cells pretreated with GA, followed by UV irradiation, the percentage of apoptotic cells in an FACS assay (Fig. 1A, lanes 2–6) and the proteolysis of PARP (Fig. 1B, lanes 2–6) were substantially enhanced. Proteolyses of PARP in GA-treated cells (Fig. 1C, filled bars) were dominating 3-fold over those of the control counterparts (open bars) at 6–9 h after UV treatment. The results suggest that Hsp90 is involved in protecting Jurkat cells from UV-irradiated apoptosis.

Reduced Expression of hsp90β Gene in UV-irradiated Jurkat Cells—The expression of hsp90β was reduced gradually at both mRNA (Fig. 2A) and protein levels in UV-irradiated Jurkat cells (Fig. 2B). Both the increased p53 expression level and its acetylation (Fig. 2B, second and third rows) indicated that p53 was activated in UV-irradiated Jurkat cells. The data suggested that the reduced expression of the hsp90β gene in UV-irradiated cells was correlated to p53.

Ectopic Wild Type p53 Represses hsp90β Gene Expression in Jurkat Cells—To elucidate the mechanism of tumor suppressor p53 on hsp90β gene expression, Jurkat cells were transfected with wild type p53 expression plasmid (pC53-SN3). With a substantial increase of p53 after transfection, Hsp90β expression in Western blotting was gradually decreased in Jurkat cells (Fig. 3A, first and second rows). In addition, a gradual increase of the proteolysis of PARP indicates the occurrence of apoptosis in the cells (Fig. 3A, third row).

Co-transfection of wild type p53 (pC53-SN3) with hsp90β reporter plasmid (hsp90β-CAT) into Jurkat cells significantly repressed hsp90β reporter gene expression to ~40% of the control in a dose-dependent manner (Fig. 3B, open bars). The
cells transfected with mutant p53<sup>V143A</sup> (pC53-SCx3) did not show any repression; on the contrary, slightly enhanced expression of the hsp90<sup>β</sup> gene was found at lower dosages of the mutant construct (Fig. 3B, filled bars).

**A Wild Type p53 BS Is a Prerequisite for Ectopic p53 and UV-irradiated Effects on the hsp90<sup>β</sup> Gene**—We have shown previously that the first intron of the hsp90<sup>β</sup> gene is essential in maintaining efficient constitutive expression and is critical for heat shock induction of the hsp90<sup>β</sup> gene (21). Comparing the CAT reporter activity of the −1039/+1531 “full-length” construct (hsp90<sup>β</sup>-CAT) with that driven by other mutant constructs of the hsp90<sup>β</sup> gene, we found that the non-translated first exon was required to yield higher expression efficiency in the CAT reporter assay (data not shown). The sequence matches the p53 consensus half-site of PuPuPuCA/TGPyPy except for the fourth and fifth nucleotides in the 5′-halfsite (39). It was thus identified within the first exon of the hsp90<sup>β</sup> gene and designated as p53 BS (Fig. 4A).

To elucidate the importance of p53 BS in the promoter region of the hsp90<sup>β</sup> gene, the core sequence CAAG (−54/+57) of the 3′-half-site for p53 binding in hsp90<sup>β</sup>-CAT was mutated to GAGG, designated as m-hsp90<sup>β</sup>-CAT. Transfection of wild type p53 was carried out as described above. It was found that ectopic wild type p53 conferred a dose-dependent reduction to the hsp90<sup>β</sup>-CAT reporter activity of some 50% (Fig. 4B, open bars), whereas no obvious effect was found with m-hsp90<sup>β</sup>-CAT (Fig. 4B, filled bars). Similar to the effect of ectopic p53, hsp90<sup>β</sup>-CAT, without mutation in the p53 BS (Fig. 4C, filled bars), was reduced to a comparable level of 50% upon UV irradiation (Fig. 4C, open bars).

These results indicated that the p53 BS in the first exon of the hsp90<sup>β</sup> gene took part in a more efficient constitutive expression of the hsp90<sup>β</sup> gene and was indispensable in the response of the gene toward UV irradiation and ectopic p53 in Jurkat cells.

**Status of Wild Type p53 Binding in the Promoter of the hsp90<sup>β</sup> Gene**—A DNA fragment of 102 bp (+8/+109) consisting of the major part of the first exon in the hsp90<sup>β</sup> gene was labeled with [<sup>32</sup>P]dCTP as W (for wild type DNA) probe or as a specific competitor without labeling in EMSA. NE prepared from either wild type p53- (pC53-SN3) or mutant p53<sup>V143A</sup>-transfected (pC53-SCx3) Jurkat cells was incubated with the W probe in vitro. Only NE from the wild type p53-transfected cells was able to bind the probe that was further identified in the supershift bands with either one of the two distinct antibodies against p53 (Fig. 5, A, left, and B, lanes 1–6). However, neither a specific band nor the supershift band could be found with NE from the cells transfected with mutant p53<sup>V143A</sup> detected with the W probe in EMSA (Fig. 5A, right). In addition, the binding of NE from cells transfected with wild type p53 could be competed out by unlabeled “w” (Fig. 5B, lane 4) but not by unlabeled “m” fragment of the 102 bp in which p53 BS was mutated (Fig. 5B, lane 5). Moreover, labeled M probe was unable to form any binding complex with the NEs from wild type-p53-transfected cells (Fig. 5B, lanes 7–9).

To further examine the status of p53 binding to the promoter...
region of the hsp90β gene in vivo, a ChIP assay with antibody against p53 was performed. We found that p53 bound to the promoter of hsp90β both constitutively and in UV-irradiated Jurkat cells (Fig. 6A, top) at an efficiency of 1.0–1.1, respectively (as measured by the ratio of the density of the IP band to that of the input band of each sample). As a positive control, the 5'-p53 BS upstream of the p21 gene was also studied (Fig. 6A, middle) and showed a constitutive binding at the p53 site of the p21 gene as reported previously (40). As expected, the amplification of the fifth exon of the hsp90β gene was unattainable in the same IP (Fig. 6A, bottom).

Differential Recruitment of p300 or mSin3a to the Promoter of hsp90β in Jurkat Cells—To further explore hsp90β expression in Jurkat cells, a ChIP assay with antibodies against p300 or mSin3a was performed. It was shown that p300 was capable of binding to the p53 BS of the hsp90β gene constitutively (Fig. 6B, lane 2 of the upper row at the top), whereas mSin3a was available only in UV-irradiated cells (Fig. 6B, lane 5 of the lower row at the top). In this context, p53 binding was a prerequisite for the regulation of the hsp90β gene that functioned in the recruiting of p300 for activation and mSin3a for repression of the gene.

DISCUSSION

It has been known that moderate cell stress alleviates the impact of damage in the cells and promotes recovery, whereas severe and sustained stressful stimuli cause apoptosis to eliminate non-repaired damaged cells. Dominant factors in each of the two functionally opposing pathways determine cell fate or susceptibility to a damage (41).

Tumor suppressor p53 mediates cell cycle arrest or apoptosis in a cellular stress response depending upon the cell type or severity of stress (9, 42). In this context, p53 may function in two ways, as either an activator or a repressor on its target genes; that is, it may activate an inhibitor or repress an activator that regulates cell cycle progression or apoptosis. p53 may bind to particular sites in the promoter regions of its target gene in a sequence-specific manner and regulate transcription of its target genes; alternatively, p53 may function via protein networks in the nucleus and in the cytoplasm as well (43).

To achieve appropriate functions in an apoptotic cell, Hsp90 has to be strictly controlled for its quantity and quality. The existing quantity of Hsp90 depends upon the balance between transcription and translational efficiency of the hsp90 gene and the stability of the product of the gene; however, the function of Hsp90 could be impaired in the presence of an inhibitor, such as GA in our system. In Jurkat cells, although a minimum level of endogenous p53 exists, Hsp90β expression is high as detected in Western blotting (Fig. 2B, lane 1). GA applied at this point does not substantially change the apoptotic markers (Fig. 1), indicating that the function of Hsp90β is not closely relevant to the onset of apoptosis of the cells. In UV irradiation-induced apoptotic Jurkat cells, however, a lowering of the expression of Hsp90 could be enhanced in GA-generated cells (Fig. 1B, lanes 4–6) implying that the antiapoptotic role of
Hsp90 could be blocked by GA. The opposite change in the cellular level of p53 and the expression of hsp90β in the UV-irradiated cells (Figs. 2 and 3) brought about the idea that p53 could be a negative regulator for hsp90β in the apoptotic cells.

To disclose the inverse correlation between p53 and hsp90β in Jurkat cells, we checked the regulatory sequences of the hsp90β gene and found a p53 BS at +31/+60 with the sequence of 5′-GGGacTGTCTnnnnnnnnAAGCAAGCCT-3′ in the first non-translated exon of the hsp90β gene (Fig. 4A). Mutation and functional studies indicate that wild type p53 can bind to the p53 BS of the hsp90β gene constitutively, and the p53 binding is essential for controlling the expression level of the gene (Figs. 4 and 5).

We have demonstrated that the wild type p53 and its BS in the first exon of the hsp90β gene are indispensable in the regulation of p53 on the hsp90β gene. First, the p53 BS in hsp90β gene is required in the constitutively efficient expression of the hsp90β gene in Jurkat cells (Fig. 4B, first group of bars from the left). Secondly, the specific and supershifted bands in EMSA (Fig. 5, A and B) only showed up in the presence of both wild type p53 and the BS of the hsp90β gene. Thirdly, the repressed expression of the hsp90β gene in a dose-dependent manner (Fig. 4B) is only shown in the cells transfected with wild type p53 with non-mutated p53 BS in the gene (Fig. 4B, open bars). Fourth, UV irradiation-reduced reporter activity driven by the hsp90β promoter can only be found in those cells with non-mutated p53 BS (Fig. 4C, open bars). We may thus draw a conclusion that p53 binds to its BS in the hsp90β gene both constitutively and in UV irradiation-treated cells (Fig. 6A), indicating that the binding is a prerequisite for the regulation of the gene.

To study whether other specific factors bind to the p53 BS to differentially regulate the hsp90β gene, we have further performed two additional ChIP assays on p300 and mSin3a. The histone acetyltransferase p300 can be recruited by a trans factor to the promoter region of a gene. It then acetylates lysine residues in the N terminus of the core histones to induce an open conformation for the gene (44, 45). In our ChIP system,
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We provide here the first evidence showing p53, as a repressor, to inhibit hsp90β expression in UV-irradiated Jurkat cells by direct binding to its BS of the gene. The reciprocity between the tumor suppressor p53 and the expression of hsp90β gene should be pivotal in determining cell fate in stress responses.

In summary, we demonstrate that wild type p53 is capable of binding to the promoter region of the hsp90β gene that confers a bihasic role to the expression of the gene in Jurkat cells. Although p53 is required for the constitutive expression of the gene in Jurkat cells by direct binding to its BS of the gene. The reciprocity between the tumor suppressor p53 and the expression of hsp90β gene should be pivotal in determining cell fate in stress responses.

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