Sensitization of TRAIL-resistant cells by inhibition of heat shock protein 90 with low-dose geldanamycin

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Abstract

Due to its specificity and effectiveness, tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL) is being tested for cancer therapy. Inhibition of the function of heat shock protein 90 (HSP90) is under clinical trials for cancer therapy. However, some cancer cells are resistant to TRAIL, and at the dose required for inducing apoptosis, geldanamycin, a drug that inhibits HSP90 function, has shown adverse effects. Therefore, our working plan was to identify a sublethal dose of geldanamycin and combine it with TRAIL to induce apoptosis in TRAIL-resistant prostate cancer cells. Treatment of LNCaP with 250 nmol/L geldanamycin inhibited HSP90 function but did not induce significant apoptosis. However, combination of geldanamycin and TRAIL induced highly significant apoptosis in TRAIL-resistant LN CaP cells. In addition to inducing caspase activity and apoptosis, treatment with geldanamycin and TRAIL decreased inhibitor of κB (IκB) kinase (IKK) complex proteins, IKKα, IKKβ, and IKKγ. The loss of IKK affected IκBα/nuclear factor-κB (NF-κB) interaction and reduced nuclear transport of NF-κB, resulting in reduced NF-κB activity. Our data show increase in apoptosis using low, suboptimal dose of geldanamycin when used with TRAIL. These results provide a means to alleviate two problems: resistance to TRAIL and adverse effects of high-dose geldanamycin.


Introduction

The American Cancer Society indicated that cancer is now the primary cause of death in America, and 30,350 people are expected to die of prostate cancer in 2005. These statistics show the enormity of the problem and the necessity of having to design new treatment options for prostate cancer. Androgen deprivation therapy has been a common treatment for prostate cancer to induce apoptosis in androgen-responsive cells (1–4). However, androgen-insensitive cells are capable of undergoing apoptosis upon appropriate stimulus (5–8), which prompted investigation of several drugs to induce apoptosis in these cells. However, the tested drugs suffer from shortcomings, such as low potency, severe adverse effects, and/or lack of specificity. Among the new generation of drugs being tested, tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL), a member of the tumor necrosis factor-α family has shown promise. The advantages are that TRAIL induces apoptosis preferentially in cancer cells, and in animal experiments, TRAIL did not show appreciable adverse effects (9, 10). Therefore, we examined the response of prostate cancer cells to TRAIL and showed that most prostate cancer cells were responsive to TRAIL, although some cells, such as LN CaP, were resistant (11–15).

Heat shock proteins (HSP) are molecular chaperones that transport and stabilize proteins within the cell. HSPs have been shown to bind to several client proteins, a necessary event for the continued function of the client proteins. Most of HSP90 client proteins are pro survival, antiapoptotic proteins, such as Akt and inhibitor of κB (IκB) kinase (IKK) complex, which are functional only when they are interacting with HSP90. Thus, continued functional integrity of HSP90 favors cell proliferation as seen by the correlation of its increased expression in cancer cells with poor prognosis and resistance to chemotherapy or radiation therapy (16). Therefore, it was hypothesized that the inhibition of the function of HSP90 would down-regulate the function of antiapoptotic proteins and promote apoptosis. The first molecule successfully used for inhibiting the function of HSP90 was benzoquinone ansamycin geldanamycin, which was later modified into 17-allylaminogeldanamycin. Both geldan amycin and 17-allylaminogeldanamycin bind to HSP90 and interfere with its interaction with client proteins. As these drugs induced cell death, they are in clinical trials for cancer therapy. However, animal experiments and clinical trials have shown that the doses of geldanamycin required for inducing apoptosis also resulted in severe adverse effects, such as hepatotoxicity (17–19). Therefore, we hypothesized that using low-dose geldanamycin would eliminate adverse effects. However, to induce effective apoptosis, it was necessary to use another apoptogenic drug, such as TRAIL.

In some cancer cells, treatment with TRAIL results in some paradoxical responses. For example, TRAIL induces apoptosis by activating the death-inducing signaling complex, leading to the activation of caspase-8 and truncation of Bid. At the same time, in some cancer cells
(typically TRAIL-resistant cells, such as LNCaP), TRAIL activates prosurvival proteins, such as nuclear factor-κB (NF-κB). NF-κB is a member of the Rel family, which consists of transcriptional activators (p65/RelA, p50/ NF-κB1, p52/ NF-κB2, RelB, and c-Rel) that form homodimers or heterodimers. These proteins are regulated by their interaction with one or more of the inhibitor subunits IκBα, IκBβ, or IκBε. In unstimulated cells NF-κB is sequestered in the cytoplasm in an inactive state due to its interaction with IκB. Upon stimulation by cytokines, IκB is phosphorylated and subsequently ubiquitinated and degraded by 26 S proteasome. NF-κB thus released from IκB is translocated into the nucleus, where it is responsible for the transcriptional activity of the target gene. NF-κB has been implicated in transformation and tumorigenesis, and recently, several laboratories have shown that NF-κB actively inhibits apoptosis by recruiting and activating antiapoptotic proteins that suppress apoptotic pathways. Thus, inhibition of NF-κB sensitizes cancer cells to apoptosis-inducing drugs (20). Furthermore, NF-κB is known to block total proteins, cytosol, or nuclear fractions were isolated as described below.

Preparation of Cell Lysates and Western Blotting
Cells were harvested by trypsinization and washed, and cell pellets were resuspended in lysis buffer (1× PBS, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 0.5 μg/μL leupeptin, 1 μg/μL pepstatin, 1 μg/μL phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin). Cells were incubated over ice for 30 minutes and centrifuged at 10,000 × g at 4°C for 10 minutes. The supernatant was collected, and the protein concentration was estimated using Bio-Rad protein reagent (Bio-Rad Laboratories, Hercules, CA).

For extracting nuclear proteins, cells were washed with cold PBS or HBSS, and the cells were released using trypsin. Cells were lysed in ice-cold lysis buffer [10 mmol/L HEPES (pH 7.9), 1.5 mmol/L MgCl₂, 10 mmol/L KCl, 0.5 mmol/L EDTA, 0.1% Triton X-100, and protease inhibitor cocktail] by passing through a No. 27 gauge needle, and the mixture was centrifuged. The supernatant was collected as cytosolic fraction, and the pellet was resuspended in nuclear extraction buffer [20 mmol/L HEPES (pH 7.9), 1.5 mmol/L MgCl₂, 0.42 mol/L NaCl, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 1.0% Igepal CA-630, 25% (v/v) glycerol, and protease inhibitor cocktail], and the membrane were disrupted using a No. 27 gauge needle. The nuclear extract was separated by centrifugation, and the supernatant was saved at −80°C until further experimentation.

Proteins (50 μg, unless stated otherwise) were separated on NuPAGE 10% Bis-Tris gels (Novex precast mini gels, Invitrogen, Carlsbad, CA) at 100 V for 1 hour in the presence of 1× MES-SDS running buffer (Invitrogen). Separated proteins were transferred to (polyvinylidene difluoride) membranes (Bio-Rad Laboratories) at 42 V for 2.5 hours using a Novex XCell II blotting apparatus in MES transfer buffer in the presence of NuPAGE antioxidant. Transfer of the proteins to the polyvinylidene difluoride membrane was confirmed by staining with Ponceau S (Sigma, St. Louis, MO). The blots were blocked in 5% nonfat dry milk in TBS, washed twice for 10 minutes each with TBS containing 0.01% Tween 20, and incubated for

Materials and Methods

Cell Culture
Prostate carcinoma cell line, LNCaP (passage 24), was obtained from the American Type Culture Collection (Rockville, MD) and were grown in RPMI 1640 supplemented with 2 mmol/L l-glutamine, 4.5 g/L glucose, 10 mmol/L HEPES, 1.5 g/L sodium bicarbonate, 1 mmol/L sodium pyruvate, and 10% fetal bovine serum (Hyclone, Logan, UT) and grown in the presence of 5% CO₂ at 37°C. Cells were treated with geldanamycin (Alexis Biochemicals, San Diego, CA) for 48 hours or with 200 ng/mL TRAIL (Biomol Research Laboratories, Inc., Plymouth Meeting, PA) for 4 hours. In combination experiments, cells were treated with geldanamycin for 44 hours, and TRAIL was added 4 hours before terminating the experiment. Upon completion of the experiments, cells were harvested, and total proteins, cytosol, or nuclear fractions were isolated as described below.
overnight at 4°C with primary antibody diluted in TBST containing 0.5% milk. The list of antibodies and their source is indicated in Table 1. Immunoreactive bands were visualized using enhanced chemiluminescence detection system (Amersham, Pharmacia Biotech, Arlington Heights, IL) on Alpha Innotech 8900 Image Analyzer. Signals were digitized, and the data were corrected for loading using the values for actin.

Measurement of Apoptosis
The M30 Apoptosense kit (Peviva AB, Bromma, Sweden) was used for measuring apoptosis. This is an ELISA that measures the generation of a neoepitope of cytokeratin 18 that is cleaved by the caspases activated in response to treatment. This assay measures early apoptotic changes and is more sensitive compared with other assays, such as Annexin V. This assay is specific to apoptosis and does not measure necrotic changes. Upon completion of the experiments, cells were harvested, and total protein was extracted as described above. Protein extract was added to 96-well plate coated with mouse monoclonal M30 antibody, and horseradish peroxidase tracer solution was added to the wells and incubated for 4 hours, as described by the supplier. Color was developed by adding tetramethyl benzidine solution, and the absorbance was determined at 450 nm on a Spectra MAX 340 microplate reader system (Amersham, Pharmacia Biotech, Arlington Heights, IL) on November 6, 2017. © 2006 American Association for Cancer Research. The signal was visualized using enhanced chemiluminescence detection microscope, and the images were captured digitally.

Transfection of NF-κB Luciferase Reporter and Luciferase Assay
LNCaP cells were grown to log phase, and 3.5 × 10^6 cells in 350 μL were mixed with luciferase reporter, pNF-κB-Luc construct containing four copies of NF-κB response element. Cells were subject to two cycles of 200 V of 10-millisecond pulse length, with a pulse interval of 500 milliseconds using Electroporator 830 (BTX, Inc., San Diego, CA). Electroporated cells were allowed to incubate in the cuvette for 15 minutes at room temperature before plating them into dishes. Cells were treated and harvested as described above and processed for the presence of luciferase activity. Luciferase activity was assayed using a kit from Promega Co. (Madison, WI). The firefly luciferase reporter assay was initiated by adding an aliquot of lysate to Luciferase Assay Reagent II, as described by the manufacturer. The assay was conducted in 96-well plates and the luminescence signals were measured (Fluorstar Optima, BMG Lab Technologies, Durham, NC). Luciferase activity was expressed per unit total protein. pEGFP-N1 vector (Biosciences, Clontech, San Jose, CA) or Renilla luciferase (Promega) was used as transfection controls.

Results
Geldanamycin Induced Apoptosis in Prostate Cancer Cells
To determine the effects of geldanamycin on prostate cancer cells, LNCaP cells were treated with increasing concentrations (range, 10–500 nmol/L) of geldanamycin. The apoptotic response of the cells was measured using M30 Apoptosense ELISA, which measures the generation of a neoepitope of cytokeratin 18 generated due to the effects of activated caspases. Results showed that at lower doses (10, 50, or 100 nmol/L) geldanamycin did...
not induce apoptosis in LNCaP cells (Fig. 1A). Increasing the dose of geldanamycin to 250 nmol/L showed marginal increase in apoptosis, whereas 500 nmol/L geldanamycin induced 7-fold apoptosis compared with controls. As our research plan was to identify a dose that is sufficient to affect HSP and HSP-related functions but not high enough to cause significant apoptosis by itself, all further experiments were conducted with 250 nmol/L geldanamycin.

Earlier reports from our and other laboratories have indicated that LNCaP are resistant to TRAIL. As one of the goals of these experiments was to determine whether disruption of the function of HSP sensitizes LNCaP to TRAIL, cells were treated with 250 nmol/L geldanamycin and increasing concentrations of TRAIL. Apoptosis assay showed no significant apoptosis until the concentration of TRAIL was raised to 200 ng/mL TRAIL, in combination with geldanamycin (Fig. 1B). Increasing the concentration of TRAIL to 400 ng/mL induced maximum apoptotic response, although no difference in apoptotic response was noted between cells treated with 200 or 400 ng/mL TRAIL. Therefore, all subsequent experiments were conducted using 200 ng/mL TRAIL and 250 nmol/L geldanamycin. Finally, combination experiments confirmed that treatment of LNCaP cells with geldanamycin and TRAIL induced synergistic apoptosis compared with geldanamycin or TRAIL alone (Fig. 1C). These experiments indicated that low-dose, subapoptotic dose of geldanamycin, which by itself did not induce significant apoptosis, sensitized TRAIL-resistant LNCaP cells.

To confirm the induction of apoptosis in these treatments, cell extracts were subject to Western blot analyses. As expected from Fig. 1, treatment with 250 nmol/L geldanamycin alone did not induce caspase-3 activity (Fig. 2A). Combination of geldanamycin and TRAIL induced significant caspase-3 activity as seen by the presence of caspase-3 cleaved products of 19, 17, and 12 kDa. When LNCaP were treated with TRAIL alone, only a 19 kDa, cleaved fragment was observed, which correlated with low level induction of apoptosis in this group (Fig. 1). Similarly, combined treatment with geldanamycin and TRAIL induced caspase-7 activity, whereas geldanamycin alone did not activate this caspase (Fig. 2A, middle). To show the effect of activated caspases on their substrate, activation of poly(ADP-ribose) polymerase was examined. As expected from apoptosense assay and caspase activation results, poly(ADP-ribose) polymerase was cleaved into 85-kDa fragment only when cells were treated with both geldanamycin and TRAIL (Fig. 2A, bottom). The above results confirmed our earlier findings that LNCaP are resistant to TRAIL and showed that low-dose geldanamycin sensitized these cells to TRAIL by activating caspases and inducing apoptosis.

The above results were obtained using LNCaP prostate cancer cells that are responsive to androgens and are wild type for the expression of antiapoptotic protein Bcl2. Therefore, it was of interest to determine the effect of geldanamycin/TRAIL on DU145 prostate cancer cells that are insensitive to androgens and do not express Bcl2. Treatment with geldanamycin or TRAIL alone induced 3-fold or 6-fold apoptosis in DU145, suggesting that
these cells are sensitive to these drugs (Fig. 1D). Combination treatment increased apoptotic response further compared with individual treatments. Furthermore, just as in LNCaP cells, combined treatment with geldanamycin and TRAIL induced significant caspase-3 activity (Fig. 2B). As the response of both the cell lines was similar, all further experiments were conducted with LNCaP cells.

Geldanamycin Sensitized LNCaP to TRAIL by Affecting the IKK Complex

Geldanamycin treatment specifically disrupts the function of HSP90, resulting in the loss of binding of HSP90 to client proteins. Published data showed that treatment of cells with geldanamycin affected the interaction between HSP90 and the IKK protein complex, leading to the loss of function of the IKK proteins and their degradation due to proteosomal activity. Therefore, experiments were conducted to determine whether geldanamycin-mediated induction of apoptosis by TRAIL is aided by the disruption of IKK proteins. As a first step, LNCaP cells were treated with increasing concentrations of geldanamycin, and the cell extracts were analyzed for the expression of the IKKα, IKKβ, and IKKγ. Results indicated dose-dependent decrease in the levels of all three IKK proteins (Fig. 3A). Reduction in IKKα levels was noticed with 50 nmol/L geldanamycin, leading to significant reduction of IKKα with 250 nmol/L geldanamycin. Further increase in the concentration of geldanamycin resulted in further loss of IKKα. It is known that the function of IKKβ and IKKγ depends upon the availability and normal function of IKKα. Similar to IKKα, IKKβ levels reduced significantly with increasing doses of geldanamycin starting with 50 nmol/L geldanamycin. However, the response of IKKβ protein to geldanamycin was significantly higher compared with IKKα, as it completely disappeared with 250 nmol/L geldanamycin. The response of IKKγ was similar to that described for IKKα and IKKβ. Thus, the levels of all three major proteins of the IKK complex were significantly reduced with increasing concentrations of geldanamycin. However, under these conditions, there was no change in the levels of HSP90 (Fig. 3A), suggesting that the reduction in the levels of IKK proteins is due to their degradation and not due to changes in the levels of HSP90 (19). Next, the effect of combined treatment with geldanamycin and TRAIL on the IKK proteins was examined. As observed above (Fig. 3A), 250 nmol/L geldanamycin reduced IKKα about 2.5-fold, whereas TRAIL did not affect IKKα (Fig. 3B). However, combination of geldanamycin and TRAIL resulted in 28-fold reduction in IKKα compared with control and 11-fold reduction compared with geldanamycin alone (Fig. 3B). Similarly, IKKβ and IKKγ showed dramatic reduction in geldanamycin-treated cells in the absence or presence of TRAIL. However, once again, under these conditions, HSP90 did not alter.

![Figure 2](Image 2.png)

Figure 2. Treatment of LNCaP cells with geldanamycin and TRAIL induced caspase activation. A, LNCaP were treated with low-dose geldanamycin (GA) and TRAIL as described in Fig. 1, and the cell extracts were analyzed by Western blots for the activation of caspase-3, caspase-7, or poly(ADP-ribose) polymerase (PARP). Blots were stripped and reprobed with antibody for actin, to be used as loading control. B, caspase-3 was analyzed by Western blots using extracts from DU145 cells. Representative data from at least three replicates.

![Figure 3](Image 3.png)

Figure 3. Treatment with increasing concentrations of geldanamycin and/or TRAIL decreased the levels of IKK proteins. A, LNCaP cells were treated with increasing concentrations of geldanamycin (GA) for 48 h, and the levels of IKKα, IKKβ, IKKγ, or HSP90 were determined by using specific antibodies. The blots were stripped and reprobed with actin for loading controls. B, cells treated with 250 nmol/L geldanamycin and/or 200 ng/mL TRAIL as described in Fig. 1. Cell extracts were analyzed for the presence of IKKα, IKKβ, and IKKγ or HSP90 by Western blots. C, to confirm the effects of treatment on the formation of IKK complex, IKKγ was immunoprecipitated, and the presence of IKKα and IKKβ in the immunoprecipitate (IP) was examined by Western blots. Representative data from at least three replicates. Con, control.
Among the IKK proteins, both IKKα and IKKβ possess catalytic subunits and therefore are involved in phosphorylation functions of the IKK complex. To maintain their function, IKK proteins have to continue to exist as a complex. We hypothesized that loss of interaction with HSF90 would affect interactions with each other and affect their function. Therefore, specific IKKγ antibody was used for immunoprecipitation, and the proteins were analyzed for the presence of other IKK proteins. Results showed significant decrease in the binding of IKKα and IKKβ to IKKγ in geldanamycin-treated cells (Fig. 3C). This effect was further increased in cells treated with both geldanamycin and TRAIL, indicating that treatment with geldanamycin affected the complex formation of the IKK proteins.

**Disruption of IKK Proteins Affected the Function of IκBα**

In unstimulated cells, IκBα binds to NF-κB, thus sequestering NF-κB in the cytoplasm. In cells stimulated with cytokines, IKK proteins are activated, which phosphorylate IκBα, releasing the NF-κB from the IκBα/NF-κB complex, which then is translocated into the nucleus. To examine whether treatment with geldanamycin and/or TRAIL affected NF-κB, nuclear transport of NF-κB was examined along with its interaction with IκBα. In cells treated with geldanamycin, NF-κB was not seen in the nuclear extract (Fig. 4, top, lanes 2 and 4), indicating its continued sequestration in the cytoplasm. These results were supported by immunoprecipitation with NF-κB, which showed continued binding of NF-κB to IκBα in geldanamycin-treated cells (Fig. 4, middle, lanes 2 and 4). Treatment with TRAIL increased the level of nuclear NF-κB (Fig. 4, top, lane 3), which correlated with reduced interaction between IκBα and NF-κB (Fig. 4, middle, lane 3). However, treatment with both geldanamycin and TRAIL decreased TRAIL-induced nuclear transport of NF-κB compared with TRAIL alone. Examination of the levels of phosphorylated IκBα in these cells showed increase in the phosphorylation of IκBα in TRAIL-treated cells (Fig. 4, bottom, lanes 3 and 4), which agreed with published data that TRAIL induced NF-κB activity in LNCaP cells. It was interesting to note that although the phosphorylation of IκBα did not decrease dramatically in geldanamycin plus TRAIL-treated cells, the nuclear level of NF-κB was reduced.

To further examine the effects of treatment on nuclear translocation of NF-κB, immunocytochemical methods were used (Fig. 5). LNCaP cells were treated as above, and the locations of NF-κB/p65 and NF-κB/p50 were examined by differential fluorescence staining and the nuclei were stained with 4',6-diamidino-2-phenylindole. Results showed that in control and low-dose geldanamycin-treated cells, NF-κB/p65 and NF-κB/p50 were limited to cytoplasm (Fig. 5). Cells treated with TRAIL alone showed nuclear staining for both NF-κB/p65 and NF-κB/p50, suggesting that TRAIL promoted nuclear translocation of NF-κB/p65 (supports the data in Fig. 4). It was of interest to note that treatment with geldanamycin inhibited TRAIL-induced transport of NF-κB/p65 and NF-κB/p50 to the nucleus. These results support the Western blot data presented above and suggest that combination treatment affected NF-κB activity.

**Treatment of LNCaP Cells with Geldanamycin Affected the Function of NF-κB**

As the above results showed that the treatment affected nuclear transport of NF-κB, experiments were conducted to examine the effect of treatment on NF-κB activity. NF-κB luciferase reporter construct containing four copies of NF-κB response elements was transfected into LNCaP and treated as above. Assay for luciferase activity showed significant reduction in the luciferase activity in geldanamycin-treated cells compared with control cells (Fig. 6). Maximum luciferase activity was noted in cells treated with TRAIL, which agreed with the results of phosphorylation of IκBα and translocation of NF-κB into the nucleus. Treatment with both geldanamycin and TRAIL reduced luciferase activity, which is significant especially compared with significantly higher activity in cells treated with TRAIL alone. The results confirm that in addition to inducing apoptosis, combination of geldanamycin and TRAIL reduced NF-κB activity significantly.

**Discussion**

Prostate cancer is a multifocal disease with clones of androgen-sensitive and androgen-refractory cells (1–4). Commonly used androgen deprivation therapies induce apoptotic cell death in androgen-sensitive cells, whereas many patients with androgen-refractory cancer die due to lack of effective chemotherapeutic agents (5–8). However,
the androgen-refractory prostate cancer cells retain the capacity to undergo apoptosis (29–31). In prostate cancer cells, apoptosis has been induced by variety of agents, such as staurosporine (31, 32), levostatin (30), thapsigargin (5), okadaic acid (33), mifepristone (13, 34), and camptothecin (35, 36). In the last few years, reports from other and our laboratory have shown induction of apoptosis in prostate cancer cells using TRAIL, although some prostate cancer cells, such as LNCaP, are resistant (10, 11, 15). We have previously shown that combined treatment of LNCaP with TRAIL and mifepristone can partially overcome the resistance to TRAIL (12). Therefore, to further improve apoptotic response of TRAIL-resistant cells, we have been exploring newer agents to act synergistically with TRAIL. In this article, we have shown increased apoptotic response of LNCaP cells to TRAIL when the function of HSP90 was inhibited.

HSPs function by binding to client proteins, thus protecting the client proteins from ubiquitin proteasome–mediated degradation. As cancer cells continue to proliferate due to the increased activity of prosurvival proteins, it was hypothesized that disruption of the interaction between HSP and client proteins would result in the degradation of the client proteins (37). For this purpose, geldanamycin and 17-allylamino geldanamycin, specific inhibitors of HSP90, are being tested as therapeutic agents (38). Although both these drugs induced apoptosis, they have shown significant adverse effects, such as hepatotoxicity, raising concerns about their feasibility as therapeutic drugs (17, 18). Therefore, we hypothesized that an effective therapeutic advantage would be to identify a combination treatment that would require lower dose of geldanamycin but still induce higher level of apoptosis. Our results show that combined treatment with TRAIL and geldanamycin induced high level apoptosis, although at this low dose, geldanamycin by itself did not induce significant apoptosis. The significance of this data is not only increased apoptosis in geldanamycin-treated and TRAIL-treated cells but also the fact that this was achieved using low, suboptimal dose of geldanamycin in LNCaP cells that are resistant to TRAIL. These results imply clinical benefits as both geldanamycin and TRAIL are being individually explored as therapeutic agents.

HSP90 affects cell survival through its effects on two different pathways: (a) interfering with apoptosis at the...
by altering the function of survival proteins, such as IKK complex. Our results showed that inhibition of HSP90 function and treatment with TRAIL affected mitochondrial functions as seen by increased activation of caspase-3, caspase-7, and their substrate poly(ADP-ribose) polymerase. In addition to direct effects on mitochondria-mediated apoptosis, we expected that inhibition of HSP90 would affect prosurvival client proteins. Dose-dependent decrease of IKKa, IKKβ, and IKKγ in geldanamycin-treated cells confirms the importance of continued binding of IKK proteins to HSP90 (19). It was interesting to note that under these conditions, the levels of HSP90 did not alter, indicating that (a) the low-dose geldanamycin used in these experiments did not affect the levels of HSP90 and that (ii) the reduction in the levels of IKK proteins was not due to reduced availability of HSP90. This suggestion was supported by immunoprecipitation data, which showed loss of interaction among IKK proteins in geldanamycin-treated cells. As IKK proteins regulate the function of NF-κB activity mediated through IκBα, our data confirm the effects of treatment on this axis. Immunocytochemistry and Western blots showed inhibition of nuclear translocation of NF-κB in geldanamycin-treated cells, which was responsible for the reduced activity of NF-κB, as measured by luciferase reporter assays.

Thus, in these experiments, we have shown that inhibition of HSP90 with low-dose geldanamycin along with treatment with TRAIL has several advantages for prostate cancer treatment. It is of particular significance that we were able to increase apoptosis while reducing the dose of geldanamycin and sensitizing TRAIL-resistant cancer cells.

References


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