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Heat shock protein-90 dampens and directs signaling stimulated by insulin-like growth factor-1 and insulin

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Abstract **Heat shock protein-90 (Hsp90) buffers cells from genetic mutations and environmental stresses. To test if this capability reflects a normal physiological function of Hsp90 to buffer cellular signals, the effects of Hsp90 inhibition were measured on activation of Akt. Inhibition of Hsp90 with geldanamycin amplified Akt phosphorylation induced by insulin-like growth factor-1 (IGF-1) or insulin, indicating that Hsp90 normally buffers these signals. Furthermore, with IGF-1 stimulation Hsp90 inhibition increased p38 activation, produced additive activation of p90RSK, and slightly increased the duration of ERK1/2 activation. Hsp90 dampened Akt signaling by facilitating phosphatase-mediated dephosphorylation of Akt. Thus, Hsp90 not only buffers the cellular effects of mutations and stresses, but also buffers the magnitude and duration of activation of proliferative and survival-promoting signaling responses.**

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1. Introduction

The chaperone protein, heat shock protein-90 (Hsp90), has recently been recognized as a cellular buffer against deleterious gene mutations and environmentally stressful conditions [1]. In organisms as disparate as flies and plants, functionally eliminating the action of Hsp90 revealed hidden genetic mutations that had been buffered by the chaperone, indicative of its crucial role in balancing stability with the capability for change [2–4]. This revelation followed the defining role of heat shock proteins in general as proteins capable of minimizing the impact of toxic environmental insults, including their namesake heat shock as well as many other potentially lethal conditions [6–8]. The capacity of Hsp90 to buffer the impact of genetic and environmental influences raised the question of whether this is a specialized function mobilized under duress, or if it is part of the normal physiological function of Hsp90 that was initially recognized by the severe outcomes that occurred following unprotected exposure to mutations or insults [2,3]. In other words, does Hsp90 buffer non-toxic cellular signals as well as those that may be toxic?

In order to address the potential buffering capacity of Hsp90 in non-toxic cellular signaling mechanisms, the present study focused on signaling leading to activation of the protein kinase Akt (also known as protein kinase B), a well-characterized client protein of Hsp90. Stimulation of a variety of receptors for growth factors, insulin, and other signaling molecules activates a phosphatidylinositol 3-kinase (PI3K)-mediated signaling pathway that leads to activation of Akt via dual phosphorylation on serine-473 and threonine-308 on Akt [9,10]. Many studies have shown that Akt is complexed with Hsp90 [11–13]. Long-term (e.g., 24 h) inhibition of Hsp90 with the Hsp90-selective inhibitor geldanamycin or its analogs [14,15] causes degradation of Akt through the proteosomal pathway [16–21]. However, previous investigations did not examine the ramifications of inhibiting Hsp90 on the immediate signals causing activation of Akt.

Therefore, this study tested if Hsp90 has a buffering capacity in receptor-stimulated activation of Akt as well as other outcomes of receptor activation. The results show that inhibition of Hsp90 amplified activation of Akt following stimulation with insulin-like growth factor-1 (IGF-1) or insulin. These results suggest that Hsp90 buffers physiological as well as toxic signaling activities.

2. Materials and methods

2.1. Cell culture and treatments

Human neuroblastoma SH-SY5Y cells and human embryonic kidney HEK293 cells were grown as described previously [22]. Cells were grown in serum-free media for 24 h prior to treatment with geldanamycin, 25 μM radicicol, 10 μM LY294002 (Alexis, San Diego, CA), 50 ng/ml IGF-1 (Serologicals, Purchase, NY), 100 nM insulin (Sigma, St. Louis, MO), or 25 μM 17-allylamino-17-demethoxygeldanamycin (17-AAG; generously provided by Dr. Robert J. Schultz, Developmental Therapeutics Program, National Cancer Institute).

2.2. Immunoprecipitation

To immunoprecipitate Akt, 3.5 μg of Akt1 antibody was incubated with 25 μl of 50% protein G–Sepharose in binding buffer (100 mM sodium phosphate, pH 7.2) for 2 h at 4 °C followed by two washes with binding buffer. The cell extract (100 μg protein) was precleared with 25 μl of 50% protein G–Sepharose for 2 h at 4 °C. The extract was centrifuged and the supernatant was incubated with the Akt1-conjugated protein G–Sepharose for 18 h at 4 °C. The immobilized immune complex was washed three times with lysis buffer, mixed with 40 μl of Laemmli sample buffer, placed in a boiling water bath for 5 min, and the samples were immunoblotted for Hsp90 and Akt.

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2.3. Immunoblotting

Cells were washed twice with phosphate-buffered saline and were lysed with 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 $20\,800 \times g$ for 15 min. Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL). Where indicated, for the preparation of mitochondria and cytosolic fractions the harvested cells were homogenized by nitrogen cavitation (200 psi, for 5 min) in a cell disruption bomb (Parr Instrument Co., Moline, IL) and fractions were prepared as previously described [22]. Samples were mixed with Laemmli sample buffer (2% SDS), placed in a boiling water bath for 5 min, proteins were resolved in SDS–polyacrylamide gels, transferred to nitrocellulose, and incubated with antibodies to phospho-Ser473-Akt, phospho-Thr308-Akt, phospho-Ser9-GSK3β, phospho-Thr180/Tyr182-p38, phospho-Ser380-PTEN (phosphatase and tensin homolog deleted on chromosome 10), phospho-Thr202/Tyr204-ERK1/2 (extracellular signal-regulated kinases 1 and 2), phospho-Ser241-PDK1 (phosphoinositide-dependent protein kinase 1), phospho-Ser380-p90RSK (Cell Signaling, Beverly, MA), heat shock factor-1 (HSF-1; NeoMarkers, Fremont, CA), total Akt (Sigma), total GSK3β (BD-PharMingen/Transduction Laboratories, San Diego, CA), or Hsp90β (Stressgen, Victoria, BC, Canada). Immunoblots were developed using horseradish peroxidase-conjugated goat anti-mouse, or goat anti-rabbit IgG, followed by detection with enhanced chemiluminescence, and the protein bands were quantitated with a densitometer.

3. Results

The transcription factor HSF-1 is a well-characterized Hsp90 client protein. When complexed with active Hsp90, HSF-1 is retained in the cytosol, but it translocates to the nucleus when Hsp90 is inhibited or recruited to bind other proteins [23,24]. Therefore, the nuclear level of HSF-1 can provide a readout of inhibition of Hsp90, and this was used to determine the rate of inhibition of Hsp90 after treating of SH-SY5Y cells with geldanamycin. In untreated SH-SY5Y cells, little HSF-1 was present in the nucleus, whereas treatment with 1 μM geldanamycin caused a rapid accumulation of HSF-1 in the nucleus (Fig. 1A). There was a large increase evident within 10 min and maximal accumulation of nuclear HSF-1 was attained after 20 to 40 min of geldanamycin treatment. This fast accumulation of HSF-1 in the nucleus confirms that the inhibition of Hsp90 by geldanamycin is rapid.

To test if Hsp90 buffers the magnitude of Akt activation by intracellular signaling, SH-SY5Y cells were stimulated with IGF-1 (50 ng/ml) to activate the PI3K/Akt signaling pathway with or without pretreatment with 10 μM geldanamycin. IGF-1 treatment substantially increased Akt phosphorylation, as indicated by large increases in the levels of both phospho-Thr308-Akt and phospho-Ser473-Akt (Fig. 1B). The dual phosphorylation of Akt occurred within 5 min of treatment with IGF-1 and remained above control levels through 20 min of incubation. The same treatment with IGF-1 after geldanamycin pretreatment resulted in much larger increases in the dual phosphorylation of Akt. Quantitative analyses showed that with geldanamycin treatment the rapid stimulation (5 min) of phospho-Thr308-Akt was nearly double the values in cells stimulated with IGF-1 alone (Fig. 1C). Similar increases caused by geldanamycin were evident 10 and 20 min after stimulation with IGF-1. Treatment with IGF-1 also stimulated an increase in the phosphorylation of the Akt substrate GSK3β on serine-9, and this was greater in cells pretreated

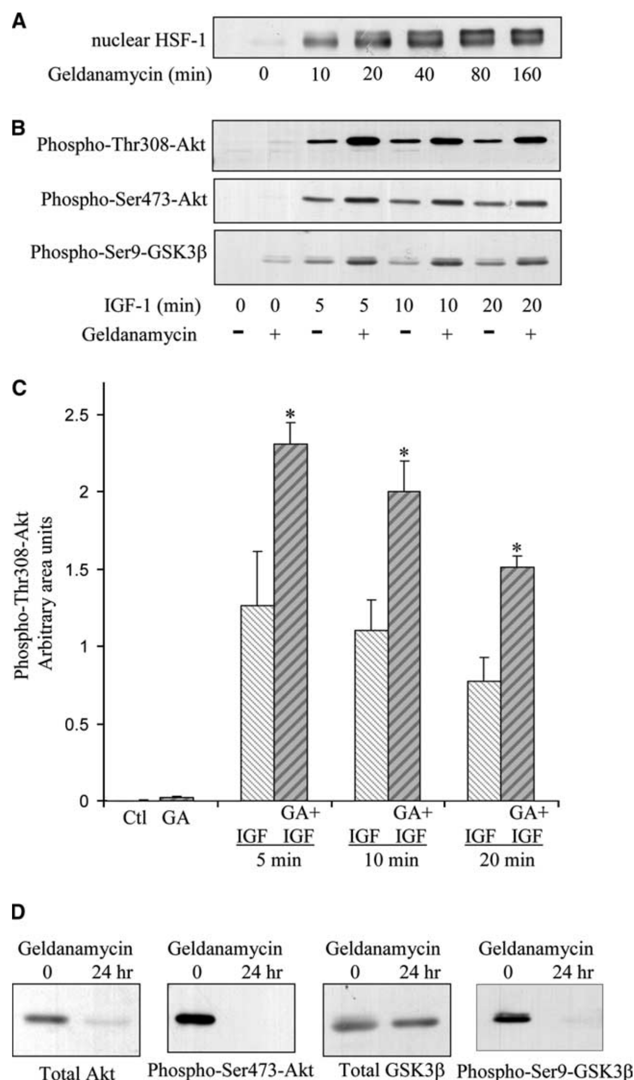

Fig. 1. Inhibition of Hsp90 by geldanamycin promotes IGF-1-induced Akt activation. (A) SH-SY5Y cells were treated with 1 μM geldanamycin for 0–160 min. Nuclear fractions were prepared and nuclear levels of HSF-1 were measured by immunoblot analysis. (B) SH-SY5Y cells were treated with 50 ng/ml IGF-1 for 0, 5, 10, or 20 min, with or without pretreatment with 10 μM geldanamycin for 30 min. Levels of phospho-Thr308-Akt, phospho-Ser473-Akt, and phospho-Ser9-GSK3β were measured by immunoblot analysis. (C) Quantitative values for phospho-Thr308-Akt were obtained from three independent experiments and are means ± S.E.M. $^*P < 0.05$. Ctl, control untreated samples; GA, geldanamycin. (D) SH-SY5Y cells were treated with 10 μM geldanamycin for 24 h followed by measurements of total Akt, phospho-Ser473-Akt, total GSK3β, and phospho-Ser9-GSK3β by immunoblot analysis.

with geldanamycin (Fig. 1B), verifying that the phosphorylated Akt was active. These results indicate that inhibition of Hsp90 allowed more Akt to be recruited and activated by the IGF-1-stimulated signaling pathway.

Contrasting with geldanamycin's facilitation of rapid signaling to Akt, long-term treatment with geldanamycin (24 h) to inhibit Hsp90 caused a large decrease in the level of Akt in SH-SY5Y cells (Fig. 1D), as reported previously in SH-SY5Y and other cell types [16,19]. With this treatment, there was also no detectable phosphorylated Akt and there was a large decrease in the level of phospho-Ser9-GSK3β, indicative of in-

activation of Akt, whereas the total level of GSK3β was unaffected, indicating that the loss of Akt stems from its role as a Hsp90 client protein. These responses demonstrate that Akt in SH-SY5Y cells responds similarly to inhibition of Hsp90 following long-term treatment with geldanamycin as has been observed in other cells.

To test if the promotion of Akt activation by geldanamycin-induced inhibition of Hsp90 is a general response, we examined another cell type, HEK 293 cells, and used a different stimulant, insulin. Treatment with insulin (100 ng/ml) caused a rapid and robust increase in the phosphorylation of Akt at both Thr308 and Ser473 in HEK cells (Fig. 2). Quantitative analysis of several experiments demonstrated that pretreatment with geldanamycin increased the insulin-stimulated Akt phosphorylation by approximately fourfold (Fig. 2). Thus, as occurred with IGF-1 stimulation in SH-SY5Y cells, inhibition of Hsp90 allowed greater activation of Akt by the insulin-stimulated signaling pathway in HEK 293 cells.

The previous results revealed that geldanamycin treatment promoted signaling to Akt induced by receptor-saturating concentrations of IGF-1 or insulin. Lower concentrations of IGF-1 were used to compare geldanamycin's ability to potentiate activation of Akt at various signal strengths. Using 5, 10, 25, or 50 ng/ml IGF-1 to stimulate SH-SY5Y cells, it was evident that even at the lowest concentration with the weakest signal, geldanamycin greatly enhanced IGF-1-induced activation of Akt (Fig. 3A). In addition to geldanamycin, two other inhibitors of Hsp90, 17-AAG and radicicol, also potentiated IGF1-induced phosphorylation of Akt and the subsequent increase in phospho-Ser9-GSK3β (Fig. 3B). Although geldanamycin inhibits Hsp90, it has been reported not to cause the dissociation of Hsp90 from Akt (11). We tested if that also held true with IGF-1 stimulation by measuring the amount of Hsp90 that co-immunoprecipitated with Akt. Equal amounts of Hsp90 co-immunoprecipitated with Akt following treatment with geldanamycin, IGF-1, or both agents (Fig. 3C), consistent with previous conclusions that Hsp90 and Akt remain in a complex regardless of the inhibitory effect of geldanamycin.

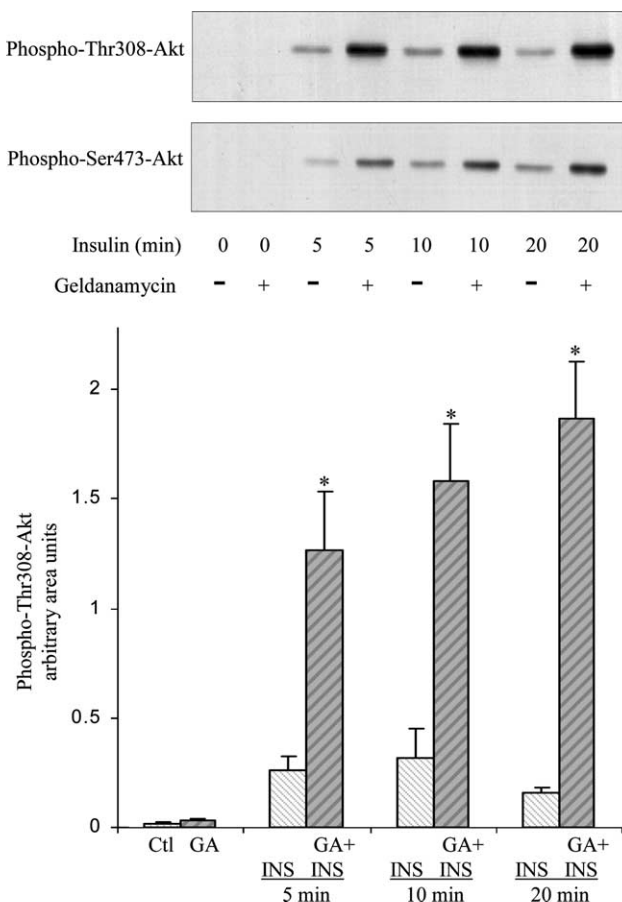

Fig. 2. Inhibition of Hsp90 by geldanamycin promotes insulin-induced Akt activation. HEK 293 cells were treated with 100 nM insulin (INS) for 0, 5, 10, or 20 min, with or without pretreatment with 10 μM geldanamycin (GA) for 30 min. Levels of phospho-Thr308-Akt and phospho-Ser473-Akt were measured by immunoblot analysis. Quantitative values for phospho-Thr308-Akt were obtained from three independent experiments and are means ± S.E.M. $^{*}P < 0.05$ compared to matched samples not treated with geldanamycin.

Although Akt is predominantly a cytosolic enzyme, we recently found that it translocates to the mitochondria following stimulation [22]. Therefore, we tested if the potentiating effect of geldanamycin was selective for either of these pools of Akt. In the cytosol, the dual phosphorylation of Akt was increased in cells treated with both geldanamycin and IGF-1 (Fig. 3D). Two effects are evident in mitochondria: the translocation of Akt into the mitochondria and the increase in Akt phosphorylation. The level of Akt in the mitochondria increased following IGF-1 treatment and this mitochondrial import of Akt was unaltered by geldanamycin pretreatment. However, after geldanamycin treatment there were large increases in both phosphorylation sites of Akt that accumulated in the mitochondria. Thus, the IGF-1-stimulated phosphorylation of both cytosolic and mitochondrial pools of Akt was increased by geldanamycin pretreatment.

The activation of Akt is regulated by PDK1 and by PTEN. Phosphorylation of threonine-308 of Akt is mediated by PDK1, a kinase thought to be constitutively active that phosphorylates Akt after Akt translocates to the 3-phosphoinositides in the plasma membrane synthesized by the action of PI3K [9]. We tested if geldanamycin treatment altered the active state of PDK1, which can be detected by immunoblot measurement of the active phospho-PDK-1. Treatment with IGF-1 alone or with geldanamycin did not change the level of phospho-PDK1 (Fig. 3E), indicating that the facilitation of Akt phosphorylation by geldanamycin was not due to increased activation of PDK1. Acting in opposition to PI3K, PTEN mediates the hydrolysis of 3-phosphoinositides, thereby reducing signaling to Akt, and is active when phosphorylated on serine 380 (Fig. 3A). As with PDK1, neither treatment with IGF-1 alone nor with geldanamycin plus IGF-1 altered the level of phospho-PTEN, indicating that increased Akt activation after geldanamycin treatment was not due to inhibition of PTEN. These results support the hypothesis that inhibition of Hsp90 does not alter the magnitude of the signal directed to Akt, but most likely reflects a direct interaction of Akt with Hsp90.

The previous results indicate that active HSP90 limits signaling-induced phosphorylation of Akt, since this response to IGF-1 or to insulin was greater following inhibition of Hsp90 with geldanamycin. Therefore, we examined if inhibition of HSP90 with geldanamycin influenced the activation of other kinases after stimulation with IGF-1, including p38, ERK1/2,

and p90RSK (Fig. 3E). Each of these proved to give a unique signature following stimulation with IGF-1 alone and in combination with geldanamycin. There was virtually no activation of p38 following stimulation with IGF-1, but in the presence of geldanamycin there was clearly strong phosphorylation of p38 stimulated by IGF-1 (Fig. 3E). The phosphorylation of p90RSK differed from the other kinases in that both IGF-1 and geldanamycin alone produced equivalent large increases, and the two together caused an approximately additive effect (Fig. 3E). ERK1/2 was rapidly phosphorylated following IGF-1 treatment, which was maximal after 5 min and then diminished after 10 and 20 min. Pretreatment with geldanamycin did not alter the maximal phosphorylation of ERK1/2 but slightly reduced the rate of dephosphorylation at 10 and 20 min after IGF-1 treatment (Fig. 3E). Thus, Hsp90 has widespread buffering effects on the activities of signaling pathways, and these vary in magnitude and duration. Thus, inhibition of Hsp90 with geldanamycin does not cause a general facilitation of IGF-1-induced signaling, but selectively promotes specific downstream signals.

Inhibition of Hsp90 with geldanamycin could increase stimulant-induced Akt phosphorylation either by blocking the dephosphorylation of phosphorylated Akt or by enhancing the phosphorylation process. To test if dephosphorylation was affected, we measured the dephosphorylation of phospho-Thr308-Akt that followed inhibition of PI3K with LY294002. Stimulation with IGF-1 activated Akt, this was potentiated by geldanamycin pretreatment, and a subsequent incubation for 2.5 min with 10 μM LY294002 caused an approximately 80% dephosphorylation of phospho-Thr308-Akt (Fig. 4A and B). Pretreatment with geldanamycin increased the level of phospho-Thr308-Akt stimulated by IGF-1, but subsequent treatment with 10 μM LY294002 caused only a 33% decline. These results indicate that inhibition of Hsp90 with geldanamycin inhibits the phosphatase-mediated dephosphorylation of phospho-Akt leading to increased levels in the presence of geldanamycin.

4. Discussion

The captivating concept of genetic buffering by Hsp90 [5] has garnered widespread experimental and theoretical support as a mechanism capable of "concealing the effects of genetic and environmental variation on phenotype" [25]. Such an intriguing concept naturally raises many questions. Among those that we found especially interesting are the questions of precisely how such buffering is achieved and whether this mechanism is somehow reserved only for aberrant signaling activities. By definition, buffering implies a dampening of the magnitudes of fluctuations. Therefore, we examined two hypotheses. First, that inhibition of Hsp90 would allow a greater magnitude of signaling activity than in cells with active Hsp90. Second, that the affected signaling need not be aberrant, as

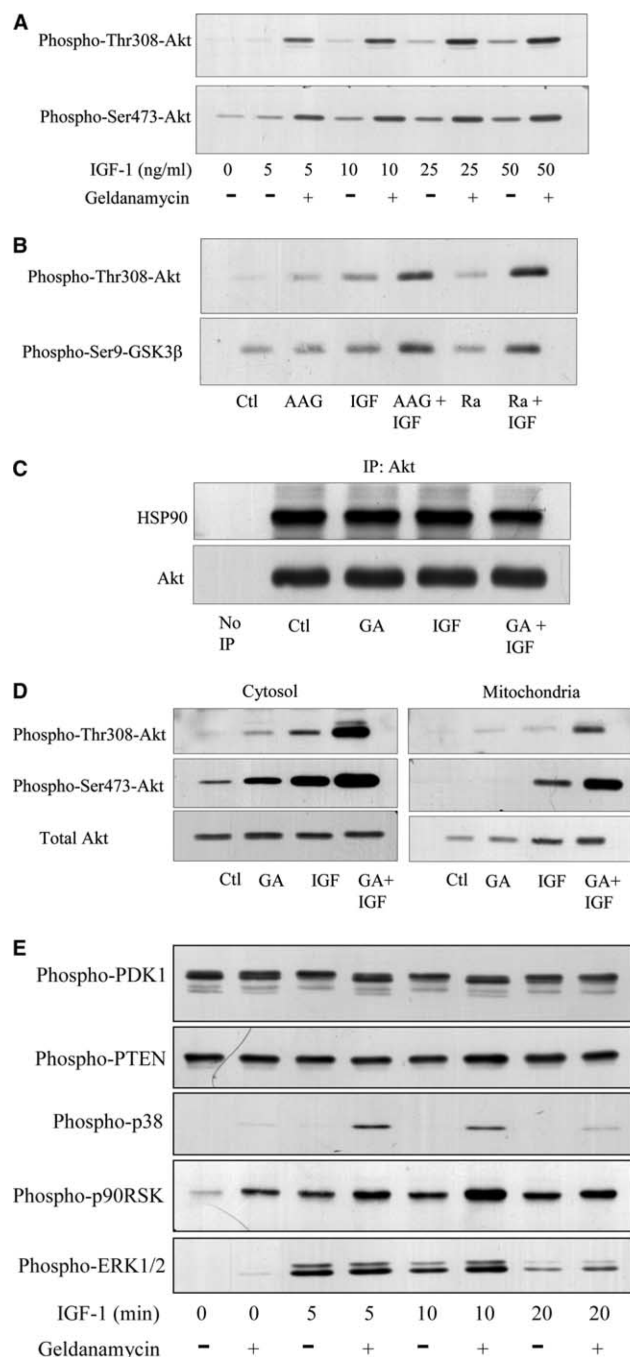

Fig. 3. Inhibition of HSP90 promotes IGF-1-induced intracellular signaling. (A) SH-SY5Y cells were pretreated with 10 μM geldanamycin for 30 min followed by stimulation for 10 min with 0, 5, 10, 25, or 50 ng/ml IGF-1. Phospho-Thr308-Akt and phospho-Ser473-Akt were examined by immunoblot analysis. (B) SH-SY5Y cells were pretreated with 25 μM 17-AAG or 25 μM radicicol (Ra) for 30 min followed by stimulation for 10 min with 5 ng/ml IGF-1 and phospho-Thr308-Akt and phospho-Ser9-GSK3β were measured. (C) SH-SY5Y cells were pretreated with 10 μM geldanamycin (GA) for 30 min followed by treatment with 50 ng/ml IGF-1 for 10 min. Akt was immunoprecipitated and the amount of Hsp90 that co-immunoprecipitated was measured. The amount of Akt that was immunoprecipitated from each sample is also shown. (D) SH-SY5Y cells were pretreated with 10 μM GA for 30 min followed by treatment with 50 ng/ml IGF-1 for 15 min. The cytosolic and mitochondrial fractions were prepared and immunoblotted for phospho-Thr308-Akt, phospho-Ser473-Akt, and total Akt. Ctl, control untreated samples. (E) SH-SY5Y cells were treated with 50 ng/ml IGF-1 for 0, 5, 10, or 20 min, with or without pretreatment with 10 μM geldanamycin for 30 min. Levels of phospho-Ser241-PDK1, phospho-Ser380-PTEN, phospho-Thr180/Tyr183-p38, phospho-Ser380-p90RSK, and phospho-Thr202/Tyr204-ERK1/2 were measured by immunoblot analyses.

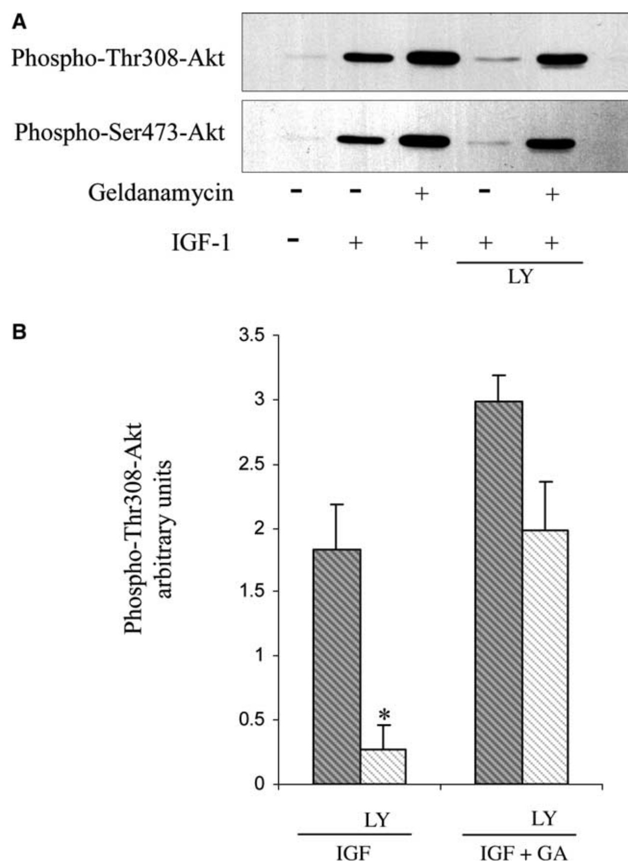

Fig. 4. Inhibition of Hsp90 blocks dephosphorylation of Akt. Cells were treated with 10 μM geldanamycin (GA) 30 min prior to stimulation with IGF-1 (50 ng/ml) for 10 min. Following IGF-1 treatment, cells were treated with 10 μM LY294002 for 2.5 min. (A) Levels of phospho-Thr308-Akt and phospho-Ser473-Akt were measured by immunoblot analyses. (B) Quantitative values for phospho-Thr308-Akt were obtained from three independent experiments and are means ± S.E.M. $^{*}P < 0.05$ compared to matched sample not treated with LY294002.

there is not an obvious mechanism by which Hsp90 could distinguish aberrant from normal signaling. To test these hypotheses, we focused on a signaling pathway that is well-characterized as providing growth- and survival-promoting cellular signals, that induced by IGF-1 or insulin leading to activation of Akt. The results show that buffering by Hsp90 can indeed be achieved by dampening the magnitude of intracellular signals and also demonstrate that this buffering action is not limited to aberrant signals.

Perhaps, the most predominant post-translational modification that is used to modulate the activity of enzymes is phosphorylation. In the case of Akt, dual phosphorylation on Thr308 and Ser473 greatly increases the catalytic activity of Akt, allowing it to efficiently phosphorylate many known substrates [9,10]. This study of Akt showed that Hsp90 limits the signaling activity of Akt by facilitating the removal of the regulatory phosphates. This was evident because inhibition of Hsp90 greatly diminished this dephosphorylation deactivating process, allowing a signal (the dual phosphorylation of Akt) of greater magnitude than that achieved in the presence of active Hsp90. There is already solid evidence that protein phosphatases associate with Hsp90 and that Hsp90 can exert a regulatory influence on their activities, since several reports have demonstrated that Hsp90 binds to a number of protein phosphatases, such as protein phosphatase 2A [26], protein phosphatase 2B [27], and protein phosphatase 5 [28]. Thus, our data indicate that Hsp90 facilitates phosphatase-mediated dephosphorylation of Akt, thereby buffering the magnitude of the phosphorylation-dependent signaling activity that can be achieved.

This regulatory interaction is very similar to the Hsp72-induced suppression of c-Jun N-terminal kinase (JNK) phosphorylation and activation that has been described in detail by Sherman and colleagues [29,30]. These investigators demonstrated that Hsp72 promotes the activity of a JNK phosphatase to limit the phosphorylation of JNK. Dissociation of Hsp72 from the phosphatase by damaged proteins inactivated the phosphatase, allowing increased phosphorylation and activation of JNK [31]. Similarly, our data indicate that Hsp90 promotes the activity of an Akt phosphatase and that inhibition of Hsp90 with geldanamycin impairs the ability of Hsp90 to promote the activity of the phosphatase, thereby allowing enhanced phosphorylation of Akt. This buffering of Akt phosphorylation could rely on an interaction between Hsp90 and a phosphatase or Hsp90 could affect the conformation of Akt to modulate its accessibility to phosphorylation or dephosphorylation.

Compared with the rapid responses of Akt-associated signaling described here in which inhibition of Hsp90 is shown to promote the phosphorylation of Akt, quite different results are obtained in studies of the longer-term effects of inhibition of Hsp90. The formation of a complex between Hsp90 and Akt was first reported by Sato and colleagues [11]. Subsequently, most research on the Hsp90–Akt interaction has focused on regulation of the stability of Akt, because inhibition of Hsp90 for several hours promoted the degradation of Akt [16]. Consequently, many reports have verified that Akt levels are downregulated following six or more hours of inhibition of Hsp90 [16–21]. However, the initial effects of inhibition of Hsp90 on intracellular signaling-induced phosphorylation of Akt remained uninvestigated, and the current results demonstrate that the initial outcome of inhibiting Hsp90 is facilitation of the stimulant-induced phosphorylation of Akt. Thus, although geldanamycin-bound Hsp90 causes destabilization of Akt, this follows a more immediate facilitation of the phosphorylation of Akt.

In addition to Hsp90 dampening signaling associated with phosphorylation of Akt, Hsp90 also was found to regulate IGF-1-induced phosphorylation of several other intracellular signaling kinases. These experiments revealed a variety of regulatory actions of Hsp90 on the magnitude and/or duration of signaling-induced phosphorylation of p38, p90RSK, and ERK1/2. While the dampening of Akt signaling by active Hsp90 was found to be due to the facilitation of dephosphorylation, the mechanisms underlying the modulation of p38, pRSK90, and ERK1/2 by Hsp90 remain to be determined. These multiple effects of Hsp90 on different kinases reveal the individuality of its modulatory influences on different intracellular signaling proteins, actions that may contribute to the capability of Hsp90 to buffer against genetic and environmental stresses by limiting the magnitude of signaling fluctuations within the cells.

The capability of Hsp90 to regulate phosphorylation of Akt reported here adds to a growing number of modulatory actions

accomplished by Hsp90 binding to client proteins, the diversity of which likely underlies its ability to buffer cellular events. Hsp90 is currently known to associate with over 100 proteins (reviewed in [32]). These proteins include kinases, receptors, transcription factors, and a variety of others. Hsp90 has multifaceted effects on its variety of client proteins. These include assistance in the maturation or stabilization of clients, such as the viral and cellular proto-oncogene Src [33,34], maintenance of clients in inactive conformations, as with glucocorticoid receptors [35], and other actions. Closest to the action reported here, inhibition of Hsp90 with geldanamycin was previously found to cause a rapid activation of the double-stranded RNA-activated kinase PKR which coincided with dissociation of Hsp90 from PKR [36], differing with the stable association of Hsp90 with Akt even after treatment with geldanamycin. Although Hsp90 limits phosphorylation of Akt, it is interesting that Hsp90 can function as a molecular scaffold to facilitate Akt-dependent phosphorylation of endothelial nitric oxide synthase [12] and of telomerase reverse transcriptase [37]. Thus, the interactions of Hsp90 and Akt are complex and include attenuated phosphorylation of Akt but facilitated stability and phosphorylation of Akt by certain targets. Thus, Hsp90 has a diverse array of functions that often serve to regulate the signaling activities of its clients, regulatory actions that may underlie its capacity to buffer genetic and environmental influences on cellular metabolism.

In summary, Hsp90 was shown to dampen signaling associated with activation of Akt as well as several other intracellular signaling kinases. These actions may contribute to the capability of Hsp90 to buffer against genetic and environmental stresses by limiting the magnitude of signaling fluctuations within cells.

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References

[1] Young, J.C., Moarefi, I. and Hartl, F.U. (2001) J. Cell Biol. 154, 267–273.
[2] Rutherford, S.L. and Lindquist, S. (1998) Nature 396, 336–342.
[3] Queitsch, C., Sangster, T.A. and Lindquist, S. (2002) Nature 417, 618–624.
[4] Milton, C.C., Huynh, B., Batterham, P., Rutherford, S.L. and Hoffmann, A.A. (2003) Proc. Natl. Acad. Sci. USA 100, 13396–13401.
[5] Sangster, T.A., Lindquist, S. and Queitsch, C. (2004) Bioessays 26, 348–362.
[6] Nollen, E.A.A. and Morimoto, R.I. (2002) J. Cell Science 115, 2809–2816.
[7] Macario, A.J.L. and de Marcario, E.C. (2000) Int. J. Clin. Lab. Res. 30, 49–66.
[8] Takayama, S., Reed, J.C. and Homma, S. (2003) Oncogene 22, 9041–9047.
[9] Brazil, D.P. and Hemmings, B.A. (2001) Trends Biochem. Sci. 26, 657–664.
[10] Brunet, A., Datta, S.R. and Greenberg, M.E. (2001) Curr. Opin. Neurobiol. 11, 297–305.
[11] Sato, S., Fujita, N. and Tsuruo, T. (2000) Proc. Natl. Acad. Sci. USA 97, 10832–10837.
[12] Fontana, J., Fulton, D., Chen, Y., Fairchild, T.A., McCabe, T.J., Fujita, N., Tsuruo, T. and Sessa, W.C. (2002) Circ. Res. 90, 866–873.
[13] Fujita, N., Sato, S., Ishida, A. and Tsuruo, T. (2002) J. Biol. Chem. 277, 10346–10353.
[14] Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E. and Neckers, L.M. (1994) Proc. Natl. Acad. Sci. USA 91, 8324–8328.
[15] Grenert, J.P., Sullivan, W.P., Fadden, P., Haystead, T.A., Clark, J., Mimnaugh, E., Krutzsch, H., Ochel, H.J., Schulte, T.W., Sausville, E., Neckers, L.M. and Toft, D.O. (1997) J. Biol. Chem. 272, 23843–23850.
[16] Basso, A.D., Solit, D.B., Chiosis, G., Giri, B., Tsichlis, P. and Rosen, N. (2002) J. Biol. Chem. 277, 39858–39866.
[17] Hostein, I., Robertson, D., DiStefano, F., Workman, P. and Clarke, P.A. (2001) Cancer Res. 61, 4003–4009.
[18] Nimmanapalli, R., O'Bryan, E., Kuhn, D., Yamaguchi, H., Wang, H.G. and Bhalla, K.N. (2003) Blood 102, 269–275.
[19] Kim, S., Kang, J., Hu, W., Evers, B.M. and Chung, D.H. (2003) Int. J. Cancer 103, 352–359.
[20] Beliakoff, J., Bagatell, R., Paine-Murrieta, G., Taylor, C.W., Lykkesfeldt, A.E. and Whitesell, L. (2003) Clin. Cancer Res. 9, 4961–4971.
[21] Doong, H., Rizzo, K., Fang, S., Kulpa, V., Weissman, A.M. and Kohn, E.C. (2003) J. Biol. Chem. 278, 28490–28500.
[22] Bijur, G.N. and Jope, R.S. (2003) J. Neurochem. 87, 1427–1435.
[23] Shi, Y., Mosser, D.D. and Morimoto, R.I. (1998) Genes Dev. 12, 654–666.
[24] Zou, J., Guo, Y., Guettouche, T., Smith, D.F. and Voellmy, R. (1998) Cell 94, 471–480.
[25] Rutherford, S.L. (2000) Bioessays 22, 1095–1105.
[26] Yoshinari, K., Kobayashi, K., Moore, R., Kawamoto, T. and Negishi, M. (2003) FEBS Lett. 548, 17–20.
[27] Someren, J.S., Faber, L.E, Klein, J.D. and Tumlin, J.A. (1999) Biochem. Biophys. Res. Commun. 260, 619–625.
[28] Silverstein, A.M., Galigniana, M.D., Chen, M.S., Owens-Grillo, J.K., Chinkers, M. and Pratt, W.B. (1997) J. Biol. Chem. 272, 16224–16230.
[29] Gabai, V.L., Meriin, A.B., Mosser, D.D., Caron, A.W., Rits, S., Shifrin, V.I. and Sherman, M.Y. (1997) J. Biol. Chem. 272, 18033–18037.
[30] Gabai, V.L. and Sherman, M.Y. (2002) J. Appl. Physiol. 92, 1743–1748.
[31] Meriin, A.B., Yaglom, J.A., Gabai, V.L., Zon, L., Ganiatsas, S., Mosser, D.D., Zon, L. and Sherman, M.Y. (1999) Mol. Cell. Biol. 19, 2547–2555.
[32] Pratt, W.B. and Toft, D.O. (2003) Exp. Biol. Med. 228, 111–133.
[33] Xu, Y. and Lindquist, S. (1993) Proc. Natl. Acad. Sci. USA 90, 7074–7078.
[34] Xu, Y., Singer, M.A. and Lindquist, S. (1999) Proc. Natl. Acad. Sci. USA 96, 109–114.
[35] Pratt, W.B. (1993) J. Biol. Chem. 268, 21455–21458.
[36] Donze, O., Abbas-Terki, T. and Picard, D. (2001) EMBO J. 20, 3771–3780.
[37] Haendeler, J., Hoffmann, J., Rahnam, S., Zeiher, A.M. and Dimmeler, S. (2003) FEBS Lett. 536, 180–186.