A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors

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Heat shock protein 90 (Hsp90) is a molecular chaperone that plays a key role in the conformational maturation of oncogenic signalling proteins, including HER-2/ErbB2, Akt, Raf-1, Bcr-Abl and mutated p53[1–7]. Hsp90 inhibitors bind to Hsp90, and induce the proteasomal degradation of Hsp90 client proteins[6,8–11]. Although Hsp90 is highly expressed in most cells, Hsp90 inhibitors selectively kill cancer cells compared to normal cells, and the Hsp90 inhibitor 17-allylaminogeldanamycin (17-AAG) is currently in phase I clinical trials[12,13]. However, the molecular basis of the tumour selectivity of Hsp90 inhibitors is unknown. Here we report that Hsp90 derived from tumour cells has a 100-fold higher binding affinity for 17-AAG than does Hsp90 from normal cells. Tumour Hsp90 is present entirely in multi-chaperone complexes with high ATPase activity, whereas Hsp90 from normal tissues is in a latent, uncomplexed state. *In vitro* reconstitution of chaperone complexes with Hsp90 resulted in increased binding affinity to 17-AAG, and increased ATPase activity. These results suggest that tumour cells contain Hsp90 complexes in an activated, high-affinity conformation that facilitates malignant progression, and that may represent a unique target for cancer therapeutics.

The naturally occurring ansamycin antibiotic geldanamycin (GM) binds to a conserved binding pocket in the amino-terminal domain of Hsp90[14,15], inhibiting ATP binding and ATP-dependent Hsp90 chaperone activity[16–18]. The GM derivative 17-AAG exerts anti-tumour activity in preclinical models[19] and is currently in clinical trials[12,20]. However, two paradoxical observations have confounded understanding of the preferential effects of Hsp90 inhibitors on tumour cells. First, it is unclear why 17-AAG binds purified Hsp90 protein with micromolar affinity *in vitro*[21] but has nanomolar activity in tissue culture[1]. Second, although Hsp90 is abundantly expressed in most cells, ansamycin drugs selectively accumulate in human tumour xenografts *in vivo*[12,22] (our unpublished observations). It has been speculated that the physicochemical properties of 17-AAG may contribute to cellular accumulation[23], but the mechanism is unknown[12]. Previous work showing that the binding affinity of GM to Hsp90 from cell lysates[1] was greater than that for the isolated protein[21] suggested to us that ansamycin binding to Hsp90 could be influenced by additional intracellular factors. Furthermore, if the unidentified factors were present to greater extent in tumour cells that normal cells, differential binding affinity might also explain the tumour selectivity of Hsp90 inhibitors.

To investigate if Hsp90 from tumour cells had a higher binding affinity to 17-AAG than that from normal cells, we performed competitive binding assays using a biotinylated GM (biotin-GM) probe (Fig. 1a). Incubation of Hsp90 from BT474 breast carcinoma cells with increasing concentrations of 17-AAG inhibited the binding of Hsp90 to biotin-GM with an IC_{50} of 6 nM, whereas Hsp90

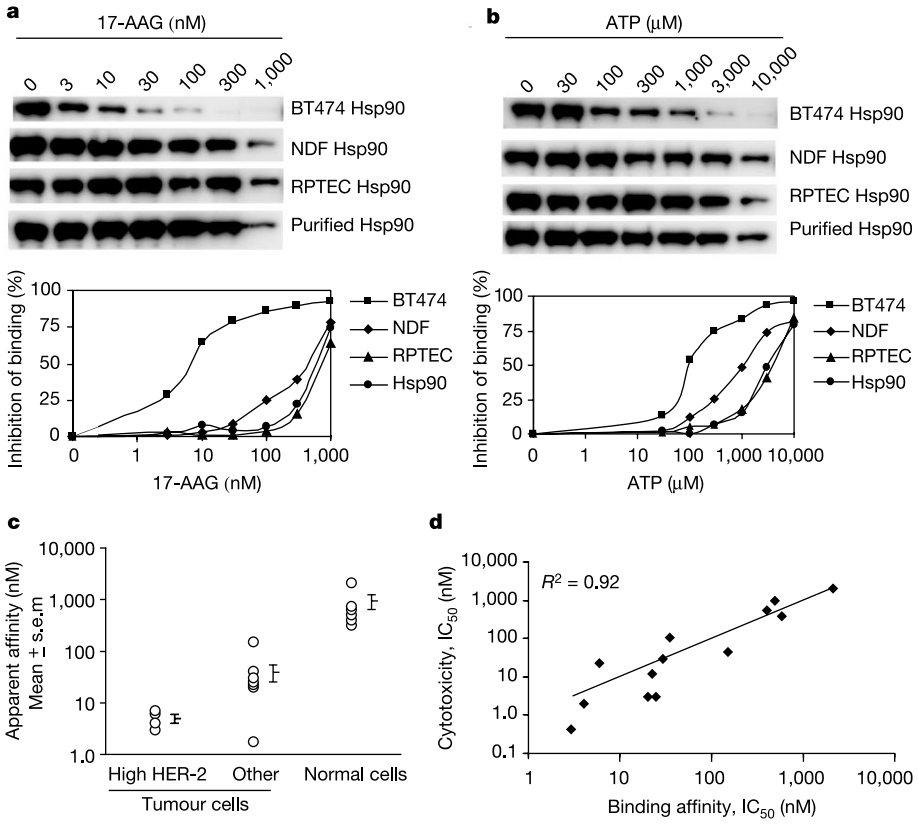

Figure 1 17-AAG has a higher binding affinity to Hsp90 from tumour cells than normal cells or purified Hsp90 protein. **a**, **b**, 17-AAG (**a**) or ATP (**b**) has a higher binding affinity to Hsp90 from BT474 tumour cells than normal cells (NDF and RPTEC) and purified Hsp90. **c**, Apparent binding affinity of 17-AAG to Hsp90 from a panel of tumour and normal cells. Individual values of IC_{50} (circles) and the mean ± standard error of the mean (line with error bars) is shown. **d**, Correlation of the binding affinity of 17-AAG to Hsp90 to the cytotoxicity in different cells.

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from primary human renal epithelial cells (RPTEC) and normal dermal fibroblasts (NDF) had an IC_{50} of 600 nM and 400 nM, respectively. Purified Hsp90 protein binding was inhibited with an IC_{50} of 600 nM, as previously reported[21]. The apparent binding affinity of ATP, the physiological ligand of Hsp90, was also higher in the tumour cell lines ($IC_{50} = 100\,\mu M$ in BT474) than the normal cells ($IC_{50} = 1{,}000\,\mu M$ in NDF and 2,900 μM in RPTEC) (Fig. 1b), whereas purified Hsp90 had an IC_{50} of 3,000 μM. In addition, by testing a large panel of cell lysates (see Methods), we observed that Hsp90 from HER-2-overexpressing cancer cells (BT474, N87, SKOV3 and SKBR3) had IC_{50} values of 5 ± 1 nM, that from other tumour cells (MCF7, A549, HT29, MDA468, SKMG3, U87, HT1080 and Hs578t) was 39 ± 14 nM, whereas Hsp90 from normal human primary cells (NDF, RPTEC, HMVEC, HMEC, HUVEC, Hs578Bst and PBMC) had IC_{50} values of 943 ± 290 nM (Fig. 1c). Total Hsp90 protein levels did not vary greatly between tumour and normal cells (Supplementary Fig. 1a). Taken together, these results suggest that the Hsp90 in tumour cells, particularly those overexpressing the Hsp90 client protein HER-2, has a significantly higher binding affinity for 17-AAG than does Hsp90 from normal cells. Furthermore, the binding affinity of 17-AAG to Hsp90 from the different cells directly correlates with the cytotoxic/cytostatic activity of 17-AAG in those cells (Fig. 1d).

Hsp90 interacts with many co-chaperone proteins that assemble into multi-chaperone complexes that promote protein folding[24]. Tumour cells overexpress Hsp90 client proteins, suggesting that a greater amount of Hsp90 in tumour cells might be engaged in active chaperoning and present in multi-chaperone complexes that could modulate the binding affinity of ligands to Hsp90. To assess whether Hsp90 in tumour cells was present in multi-chaperone complexes, we determined the levels of the co-chaperones p23 and Hop—essential components of the two known multi-chaperone Hsp90 complexes[13]—associated with Hsp90 from normal and tumour cells (Fig. 2a). Co-immunoprecipitation in the normal and tumour cell lysates with antibodies to Hsp90 revealed that more Hsp90 in tumour cells was present in complexes with p23 and Hop compared to normal cells (Fig. 2a). Control immunoprecipitations without antibodies did not immunoprecipitate any Hsp90. Strikingly, immunoprecipitation with antibodies to both p23 and Hop revealed that the entire tumour cell pool of Hsp90 was present in complexes unlike normal cells. Conversely, immunoprecipitation with an antibody[25] specific for the uncomplexed form of Hsp90 pulled down far more Hsp90 from normal cells than from tumour cells. Neither the total levels of p23 and Hop nor the growth rate of the tumour cells differed significantly from the normal cells (Supplementary Fig. 1b). These results suggest that all Hsp90 in tumour cells is in the form of multi-chaperone complexes that are actively engaged in chaperoning client oncoproteins, and that the heightened complex formation is not due to increased expression of Hsp90 or co-chaperones. As the chaperone function of Hsp90 is dependent upon its ATPase activity[16], we immunoprecipitated Hsp90 from cell lysates and performed ATPase assays. Tumour Hsp90 had markedly higher ATPase activity compared to Hsp90 from normal cells (Fig. 2b) and was inhibited by 17-AAG, radicicol (another structurally unrelated Hsp90 inhibitor), and by 0.5 M KCl (which is known to break apart chaperone complexes). Control immunoprecipitation in the absence of Hsp90 antibody did not have any significant ATPase activity (data not shown). Combined, these results suggest that essentially all soluble Hsp90 in tumour

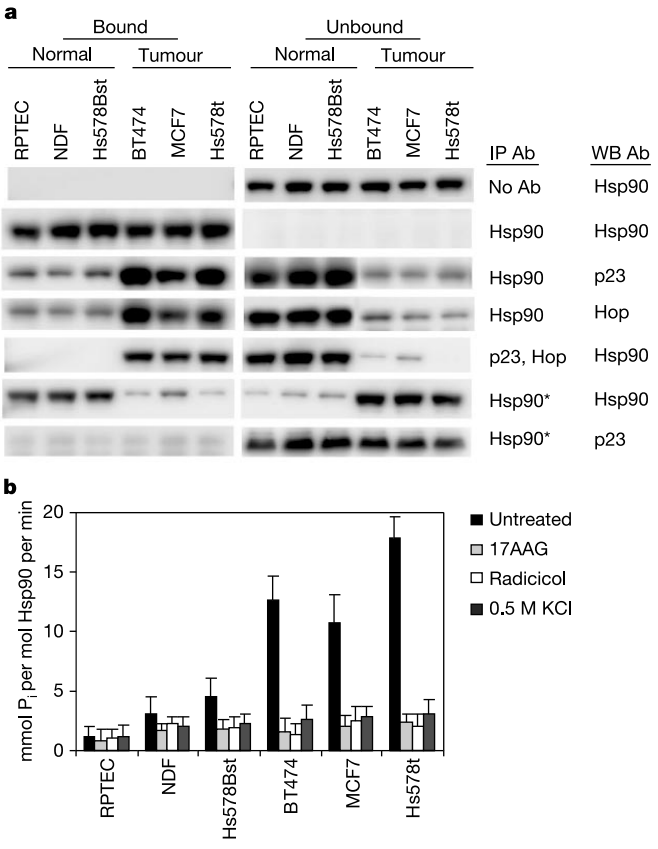

Figure 2 Hsp90 from tumour cells is present in chaperone complexes with high ATPase activity. **a**, Normal (RPTEC, NDF and Hs578Bst) and tumour (BT474, MCF7 and Hs578t) cell lysates were immunoprecipitated with indicated antibodies (IP Ab) and immunoblotted with indicated western blot (WB) Ab. The bound and unbound fractions are shown. Hsp90* antibody preferentially recognizes uncomplexed Hsp90. **b**, ATPase activity of Hsp90 from normal (RPTEC, NDF and Hs578Bst) and tumour (BT474, MCF7 and Hs578t) cell lysates that were untreated or treated with 10 μM 17-AAG, 10 μM radicicol and 0.5 M KCl. Mean ± standard deviation are shown from triplicate experiments.

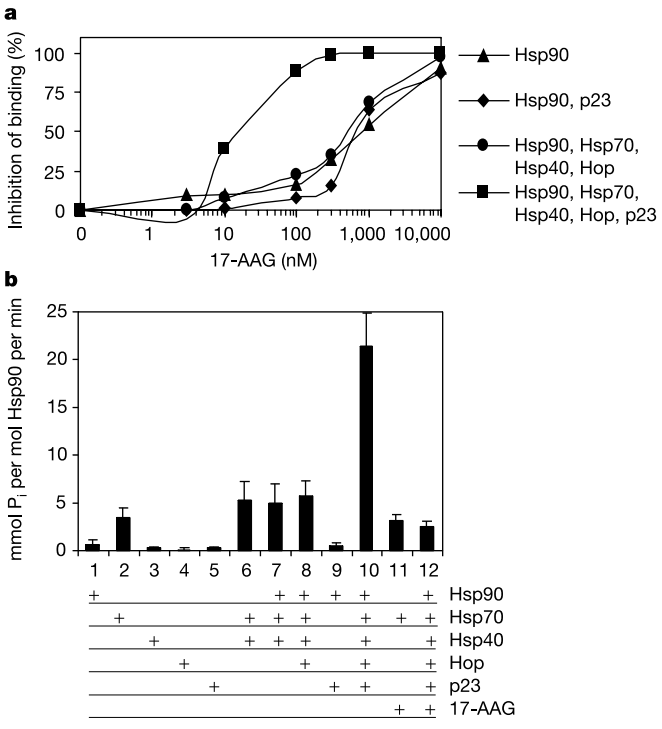

Figure 3 *In vitro* reconstitution of purified Hsp90 with co-chaperones increased binding affinity to 17-AAG and the ATPase activity. **a**, Hsp90 reconstituted with the indicated co-chaperones has a higher apparent binding affinity to 17-AAG. **b**, Hsp90 reconstituted with the indicated co-chaperones has increased ATPase activity, which can be inhibited by 10 μM 17-AAG.

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cells is present in fully active multi-chaperone complexes, whereas Hsp90 in normal cells is in an uncomplexed inactive form.

As our data demonstrated that the Hsp90 from tumour cells had a higher binding affinity to 17-AAG, and this correlates with presence of increased multi-chaperone complexes and increased ATPase activity, we tested whether reconstitution of purified Hsp90 with co-chaperones would result in increased binding affinity and ATPase activity. For *in vitro* reconstitution, we used five proteins (Hsp90, Hsp70, Hsp40, Hop and p23) that have been shown to be required for the *in vitro* chaperoning activity of Hsp90[26]. Addition of all four co-chaperones to purified Hsp90 increased the apparent affinity of 17-AAG from 600 nM for Hsp90 alone to 12 nM in the presence of the added proteins, whereas partial complexes did not (Fig. 3a). Therefore, Hsp90 when reconstituted with co-chaperones has nanomolar binding activity, in concordance with that observed in tumour cell lysates (Fig. 1). The ATPase activity of Hsp90 was also significantly enhanced by all four co-chaperones and was inhibited by the addition of 17-AAG (Fig. 3b). Hsp70 had some minimal ATPase activity, but this was not inhibited by 17-AAG. Thus, these results suggest that Hsp90 present in multi-chaperone complexes not only had a higher binding affinity for 17-AAG but was also more biochemically active.

To determine if our observations *in vitro* also applied to mice and to clinical cancer, we examined the binding affinity of 17-AAG to Hsp90 in normal and malignant mouse and human tissue samples (Fig. 4a, b). The apparent binding affinity of 17-AAG to Hsp90 from mouse tumours (3T3-src, B16 and CT26) was 8–35 nM compared to 200–600 nM for the mouse normal tissues (brain, kidney, liver, lung and heart), even though Hsp90 was more abundantly expressed in several of the normal tissues (Fig. 4a). For the human tissues, using four samples of each tissue type, the IC_{50} was 6,170 ± 1,060 nM for normal breast versus 29 ± 4 nM for breast carcinoma, and 2,725 ± 736 nM for normal colon versus 43 ± 4 nM for the colon carcinomas (Fig. 4b). To determine the fraction of Hsp90 present as multi-chaperone complexes, we performed co-immunoprecipitations and observed that there were increased amounts of p23 with Hsp90 from the human tumours compared to the normal tissues (Fig. 4c). Conversely, there was significantly more uncomplexed Hsp90 co-immunoprecipitated with the Hsp90* antibody from the normal tissues compared to tumour tissues (Fig. 4c). Furthermore, the Hsp90 ATPase activity in both clinical tumour samples was significantly higher relative to the normal tissues and was inhibited by 17-AAG, radicicol and 0.5M KCl (Fig. 4d). These results indicate that the Hsp90 in human clinical tumour tissues is in a high-affinity, multi-chaperone complex with increased ATPase activity.

Our data show that Hsp90 in tumour cells exists in a functionally distinct molecular form, and clarify three fundamental aspects of the role of Hsp90 in tumour biology. First, the nanomolar binding of 17-AAG to high-affinity tumour Hsp90 is now consistent with the nanomolar anti-tumour activity of this family of drugs. Second, the markedly higher affinity of tumour Hsp90 *in vivo* explains the remarkable ability of ansamycins to accumulate progressively at tumour sites in animals. And third, the complete usage of tumour Hsp90 may provide a selection pressure leading to further upregulation of Hsp90 that is observed in many advanced tumours[27,28]. We propose a model of Hsp90-dependent malignant progression in which, as tumour cells gradually accumulate mutant and over-expressed signalling proteins, Hsp90 becomes engaged in active chaperoning and stabilization of oncoproteins, and adopts a high-affinity form induced by bound co-chaperone proteins. Thus, Hsp90 may serve an analogous role in the somatic evolution of tumours as in darwinian evolution[29,30]—rescuing potentially misfolded mutant proteins to prevent the mutation becoming lethal to

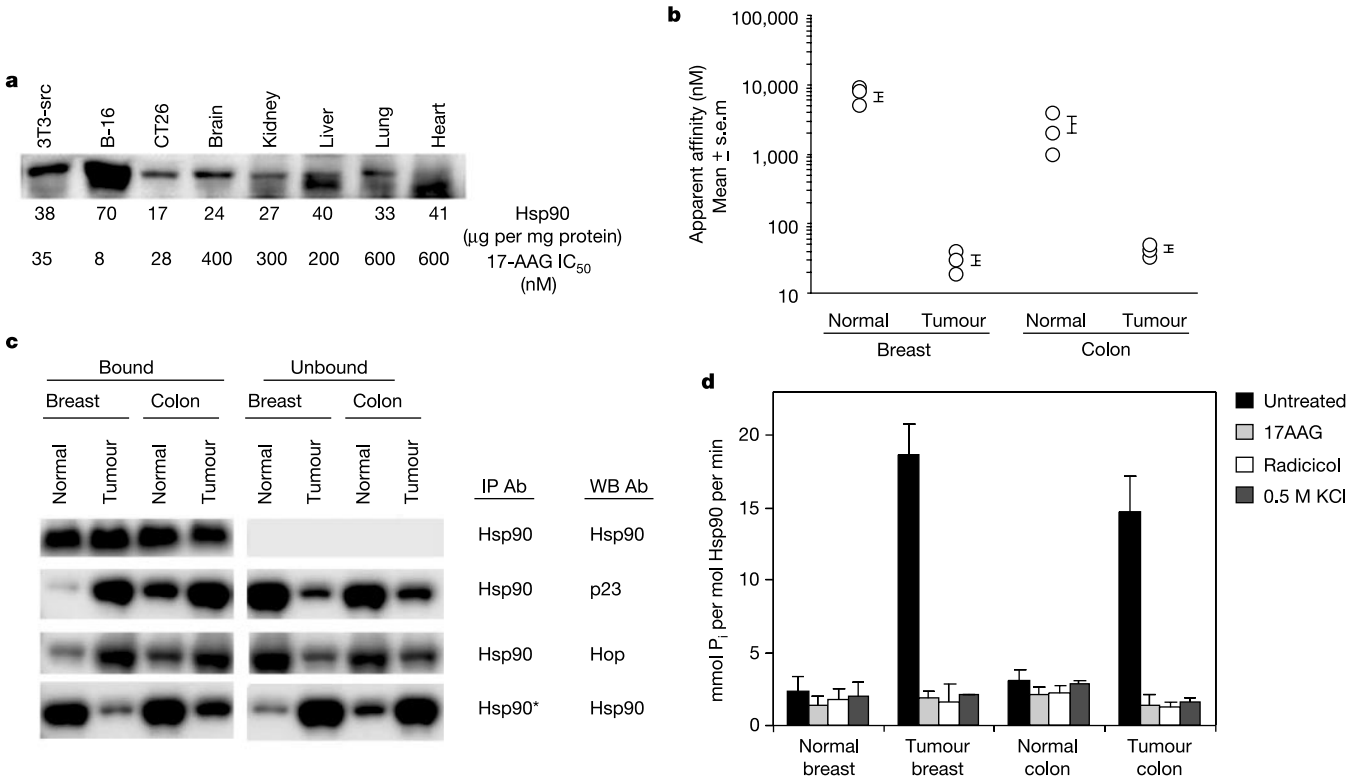

Figure 4 Hsp90 from clinical tumour samples is in a high-affinity complex with increased ATPase activity. **a**, Hsp90 protein levels and apparent binding affinity of 17-AAG to Hsp90 from mouse tumour and normal tissues. **b**, Apparent binding affinity of 17-AAG to Hsp90 from human normal and tumour tissues. Mean ± standard error is shown ($N = 4$). **c**, Human normal and tumour tissue lysates co-immunoprecipitated with Hsp90 antibodies and immunoblotted with indicated WB Ab. **d**, Hsp90 ATPase activity from human normal and tumour tissues treated with 10 μM 17-AAG or radicicol and 0.5 M KCl. Mean ± standard error is shown ($N = 4$).

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the cell, and thus allowing these oncoproteins to support tumour cell proliferation, survival and malignancy. Dependence on the activated, high-affinity chaperone could make Hsp90 an 'Achilles heel' of tumour cells, driving the selective accumulation and bioactivity of pharmacological Hsp90 inhibitors, and making tumour Hsp90 a unique cancer target. □

Methods

Cells and reagents

Normal and malignant cell lines were obtained from Clonetics and ATCC unless otherwise indicated. Tumour cells used included high-HER-2 breast carcinoma (BT474, SKBR3), stomach carcinoma (N87) and ovarian carcinoma (SKOV3), MCF7 breast carcinoma, MDA468 breast carcinoma, Hs578T breast carcinoma and paired normal epithelium Hs578Bst, A549 lung carcinoma, HT29 colon carcinoma, U87 glioblastoma, SKMG3 glioblastoma (a gift from C. Thomas) and HT1080 fibrosarcoma. Normal primary cells used were renal proximal epithelial cells (RPTEC), normal dermal fibroblasts (NDF), human microvascular endothelial cells (HMVEC), human mammary epithelial cells (HMEC), human umbilical vascular endothelial cells (HUVEC) and peripheral blood mononuclear cells (PBMC). Normal mouse tissue (brain, kidney, liver, lung and heart) was obtained from nude mice and the mouse tumours were from nude mice injected with 3T3-src (a gift from L. Neckers), B16 and CT26 cells. The clinical samples (benign breast tissue, metastatic ductal breast carcinoma, normal colonic mucosa and colon adenocarcinoma) were obtained from Bainbridge Genomic Foundation and BioResearch Support, Inc. Antibodies used were: Hsp90 (Stressgen, SPA-845; recognizes Hsp90α and Hsp90β and immunoprecipitates free and complexed Hsp90), Hsp90* (Stressgen, SPA-830; recognizes Hsp90α and Hsp90β and immunoprecipitates uncomplexed Hsp90), p23 (Alexis Biochemicals, 804-023-R100) and Hop (a gift from D. Toft). 17-AAG was synthesized from GM, and the biotin-GM probe was prepared by displacing the 17-methoxy of GM with a biotinyl-linked amine.

Hsp90 binding assays

Purified native Hsp90 protein (Stressgen) or cell lysates in lysis buffer (20 mM HEPES, pH 7.3, 1 mM EDTA, 5 mM $MgCl_2$, 100 mM KCl) were incubated with or without 17-AAG for 30 min at 4 °C, and then incubated with biotin-GM linked to BioMag streptavidin magnetic beads (Qiagen) for 1 h at 4 °C. Tubes were placed on a magnetic rack, and the unbound supernatant removed. The magnetic beads were washed three times in lysis buffer and heated for 5 min at 95 °C in SDS–PAGE sample buffer. Samples were analysed on SDS protein gels, and western blots done using indicated antibodies. Bands in the western blots were quantified using the Bio-rad Fluor-S MultiImager, and the percentage inhibition of binding of Hsp90 to the biotin-GM was calculated. The IC_{50} reported is the concentration of 17-AAG needed to cause half-maximal inhibition of binding. For *in vitro* reconstitution, 5 μM of purified Hsp90 (Stressgen) was combined with 1 μM each of Hsp70, Hsp40, p23 and Hop purified proteins (a gift from D. Toft) as previously described[26].

MTS assay

Cells were seeded in 96-well plates at 2,000 cells per well in a final culture volume of 100 μl for 24 h before the addition of increasing concentrations of 17-AAG that was incubated for 5 days. Viable cell number was determined using the Celltiter 96 AQueous Non-radioactive Cell Proliferation Assay (Promega). The value of the background absorbance at 490 nm (A_{490}) of wells not containing cells was subtracted. Percentage of viable cells = (A_{490} of 17-AAG treated sample/A_{490} untreated cells) × 100. The IC_{50} was defined as the concentration that gave rise to 50% viable cell number.

Co-immunoprecipitation

Cell lysates were prepared as described above. Protein-A Sepharose beads (Zymed) were pre-blocked with 5% BSA. The cell lysates were pre-cleared by incubating with 50 μl of protein-A Sepharose beads (50% slurry). To 100 μl of the pre-cleared cell lysate, either no antibody or antibodies to Hsp90, p23 and Hop were added, and incubated by rotating for 1 h at 4 °C. 50 μl of pre-cleared beads (50% slurry) was then added and incubated by rotating for 1 h at 4 °C. Bound beads were briefly centrifuged at 3,000*g* and unbound samples collected. Beads were washed thrice in lysis buffer and once with 50 mM Tris, pH 6.8, and then SDS-sample buffer added for 5 min at 95 °C. Bound and unbound samples were analysed by SDS–PAGE and western blots using indicated antibodies.

Hsp90 ATPase assay

Hsp90 was co-immunoprecipitated from normal and tumour cell lysates as described above using an N-terminal Hsp90 antibody. For *in vitro* reconstitution, 5 μM Hsp90 was combined with 1 μM each of Hsp70, Hsp40, p23 and Hop purified proteins[25]. The ATPase activity of the immunoprecipitated or *in vitro* reconstituted Hsp90 was measured by detection of free inorganic phosphate (P_i) using a PiPer Phosphate Assay kit (Molecular Probes). The assay measures an increase in fluorescence absorption of an Amplex Red reagent that is proportional to the amount of P_i in the sample. Standards were done using known concentrations of P_i (100 μM with six half-log dilutions), and the amount of P_i released from Hsp90 in the cell lysates or in the *in vitro* reconstituted proteins was calculated. For the cell lysates, the amount of Hsp90 immunoprecipitated with the Hsp90 antibody was quantified to normalize the data to mmol Pi per mol Hsp90 per min. All assays were done in triplicate.

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1. Schulte, T. W. & Neckers, L. M. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother. Pharmacol.* **42,** 273–279 (1998).
2. Xu, W. *et al.* Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J. Biol. Chem.* **276,** 3702–3708 (2001).
3. Sato, S., Fujita, N. & Tsuruo, T. Modulation of Akt kinase activity by binding to Hsp90. *Proc. Natl Acad. Sci. USA* **97,** 10832–10837 (2000).
4. Basso, A. D., Solit, D. B., Munster, P. N. & Rosen, N. Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. *Oncogene* **21,** 1159–1166 (2002).
5. Schulte, T. W., Blagosklonny, M. V., Ingui, C. & Neckers, L. Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. *J. Biol. Chem.* **270,** 24585–24588 (1995).
6. An, W. G., Schulte, T. W. & Neckers, L. M. The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210bcr-abl and v-src proteins before their degradation by the proteasome. *Cell Growth Differ.* **11,** 355–360 (2000).
7. Blagosklonny, M. V., Toretsky, J. & Neckers, L. Geldanamycin selectively destabilizes and conformationally alters mutated p53. *Oncogene* **11,** 933–939 (1995).
8. Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E. & Neckers, L. M. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *Proc. Natl Acad. Sci. USA* **91,** 8324–8328 (1994).
9. Miller, P. *et al.* Depletion of the erbB-2 gene product p185 by benzoquinoid ansamycins. *Cancer Res.* **54,** 2724–2730 (1994).
10. Mimnaugh, E. G., Chavany, C. & Neckers, L. Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. *J. Biol. Chem.* **271,** 22796–22801 (1996).
11. Schulte, T. W., An, W. G. & Neckers, L. M. Geldanamycin-induced destabilization of Raf-1 involves the proteasome. *Biochem. Biophys. Res. Commun.* **239,** 655–659 (1997).
12. Workman, P. Auditing the pharmacological accounts for hsp90 molecular chaperone inhibitors: Unfolding the relationship between pharmacokinetics and pharmacodynamics. *Mol. Cancer Ther.* **2,** 131–138 (2003).
13. Isaacs, J. S., Xu, W. & Neckers, L. Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell* **3,** 213–217 (2003).
14. Prodromou, C. *et al.* Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90,** 65–75 (1997).
15. Stebbins, C. E. *et al.* Crystal structure of an Hsp90-geldanamycin complex: Targeting of a protein chaperone by an antitumor agent. *Cell* **89,** 239–250 (1997).
16. Obermann, W. M., Sondermann, H., Russo, A. A., Pavletich, N. P. & Hartl, F. U. In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* **143,** 901–910 (1998).
17. Panaretou, B. *et al.* ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. *EMBO J.* **17,** 4829–4836 (1998).
18. Grenert, J. P., Johnson, B. D. & Toft, D. O. The importance of ATP binding and hydrolysis by hsp90 in formation and function of protein heterocomplexes. *J. Biol. Chem.* **274,** 17525–17533 (1999).
19. Solit, D. B. *et al.* 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Clin. Cancer Res.* **8,** 986–993 (2002).
20. Blagosklonny, M. V. Hsp-90-associated oncoproteins: Multiple targets of geldanamycin and its analogs. *Leukemia* **16,** 455–462 (2002).
21. Chiosis, G. *et al.* A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. *Chem. Biol.* **8,** 289–299 (2001).
22. Egorin, M. J. *et al.* PC3 human prostate xenograft retention of, and oncoprotein modulation by 17-(allylamino)-17-demothoxygeldanamycin(17AAG) in vivo. *Proc. Am. Assoc. Cancer Res.* **40,** 3409 (1999).
23. Chiosis, G. *et al.* 17AAG: Low target binding affinity and potent cell activity-finding an explanation. *Mol. Cancer Ther.* **2,** 123–129 (2003).
24. Richter, K. & Buchner, J. Hsp90: Chaperoning signal transduction. *J. Cell. Physiol.* **188,** 281–290 (2001).
25. Schuh, S. *et al.* A 90,000-dalton binding protein common to both steroid receptors and the Rous sarcoma virus transforming protein, pp60v-src. *J. Biol. Chem.* **260,** 14292–14296 (1985).
26. Kosano, H., Stensgard, B., Charlesworth, M. C., McMahon, N. & Toft, D. The assembly of progesterone receptor-hsp90 complexes using purified proteins. *J. Biol. Chem.* **273,** 32973–32979 (1998).
27. Gress, T. M. *et al.* Differential expression of heat shock proteins in pancreatic carcinoma. *Cancer Res.* **54,** 547–551 (1994).
28. Yano, M., Naito, Z., Tanaka, S. & Asano, G. Expression and roles of heat shock proteins in human breast cancer. *Jpn J. Cancer Res.* **87,** 908–915 (1996).
29. Rutherford, S. L. & Lindquist, S. Hsp90 as a capacitor for morphological evolution. *Nature* **396,** 336–342 (1998).
30. Queitsch, C., Sangster, T. A. & Lindquist, S. Hsp90 as a capacitor of phenotypic variation. *Nature* **417,** 618–624 (2002).

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Competing interests statement The authors declare competing financial interests: details accompany the paper on **www.nature.com/nature**.

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