Identification of new biomarkers for clinical trials of Hsp90 inhibitors

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Abstract
The selective heat shock protein 90 (HSP90) inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) is currently in phase I/II clinical studies at numerous institutions. Heretofore, the biomarkers to detect 17-AAG bioactivity (Hsp70, Raf-1, and cyclin-dependent kinase 4) had to be analyzed by Western blot of cellular samples, either from tumor biopsies or peripheral blood leukocytes, a method that is both laborious and invasive. We have identified two new biomarkers [insulin-like growth factor binding protein-2 (IGFBP-2) and HER-2 extracellular domain] that can be readily detected in patient sera by ELISA. Both secreted proteins are derived from or regulated by Hsp90 client proteins, raising hopes that they might be sensitive serum markers of HSP90 inhibitor activity. Several structurally unrelated HSP90 inhibitors dose-dependently decreased secretion of both IGFBP-2 and HER-2 extracellular domain into culture medium, and both proteins were more sensitive to HSP90 inhibitors than previously identified biomarkers. In sera from BT474 tumor-bearing mice, both IGFBP-2 and HER-2 extracellular domain were down-regulated by 17-AAG in a time-dependent and dose-dependent manner, coincident with the degradation of HER-2 and attenuation of AKT activity in the tumors. Furthermore, IGFBP-2 levels at the end of treatment correlated with residual tumor load, suggesting that IGFBP-2 might serve as an early indicator of therapeutic response. In addition, we also found that both IGFBP-2 and HER-2 extracellular domain levels are elevated in patient sera from several cancer types, suggesting that these novel secreted biomarkers could be valuable pharmacodynamic tools in clinical trials of HSP90 inhibitors. [Mol Cancer Ther 2006;5(5):1256–64]

Introduction
Heat shock protein 90 (HSP90) is a conserved molecular chaperone that mediates the maturation and stability of a set of cancer-associated proteins, collectively referred to as "clients" (1–3). These include steroid receptors, epidermal growth factor receptor family members, IGFR, MET, Raf-1, AKT, Bcr-abl, mutant p53, cyclin-dependent kinase 4, and many other oncogenic molecules. Inhibition of HSP90 causes simultaneous degradation of client proteins via an incompletely understood mechanism and results in suppression of tumor growth (1–3).

17-Allylamino-17-demethoxygeldanamycin (17-AAG) is a geldanamycin derivative HSP90 inhibitor currently in phase II trials. In phase I, the pharmacodynamic activity of the drug was evaluated by measuring Hsp70 activation and/or degradation of cyclin-dependent kinase 4 or Raf-1 (4, 5). The study had to be done by Western blot of samples from either tumor biopsies or peripheral blood mononuclear cells (PBMC), which is both laborious and time consuming. In addition, tumor biopsies are only available from a minority of patients, whereas PBMCs represent at best a surrogate for the tumor, and their use for longitudinal studies may be somewhat limited due to the relatively large amount of blood required.

In search of convenient biomarkers for Hsp90 inhibitor clinical trials, we have identified two serum proteins [insulin-like growth factor binding protein-2 (IGFBP-2) and HER-2 extracellular domain] that are derived from or are regulated by Hsp90 client proteins. IGFBP-2 is a member of IGFBP family, which exist in extracellular fluid and serum (6). The protein is about 34-36 kDa and is expressed in a number of tissues, with the highest level seen in the central nervous system (7). Binding of IGFBP-2 to IGFs facilitates the transport of IGFs and modifies the action of the growth factor on target cells. The level of IGFBP-2 is found to be elevated in a variety of cancers (8–11). Cancer cells may control cell proliferation and tumor growth by producing IGFBPs that enhance the function of IGF-I and IGF-II (6, 12, 13). IGFBP-2 is activated by a variety of pathways, such as IGFR → phosphatidylinositol 3-kinase → AKT and estrogen receptor signaling, that are regulated by Hsp90 (1). Therefore, Hsp90-dependent degradation of clients upstream of IGFBP-2 could cause a subsequent down-regulation of IGFBP-2 in serum.

Circulating HER-2 comprises the extracellular domain of HER-2. Full-length HER-2 is cleaved by a sheddase near the transmembrane domain to generate a 110-kDa fragment that is detectable in serum of most individuals and in the culture medium of HER-2-overexpressing cells (14, 15). Elevated HER-2 extracellular domain can

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be detected in the serum of patients with high HER-2-overexpressing tumors and is correlated with poor prognosis and reduced response to chemotherapy (16–18). Here, we report the effect of Hsp90 inhibition on the expression of IGFBP-2 and HER-2 extracellular domain in vitro and in vivo. 17-AAG and a panel of novel purine-based Hsp90 inhibitors were employed. 17-AAG and the Conforma compounds suppressed both secreted proteins in a dose-dependent and time-dependent manner in breast cancer cells and in human tumor xenografts. The decrease of IGFBP-2 and HER-2 extracellular domain was observed at lower drug concentrations than that required for the degradation of intracellular client proteins, suggesting that these biomarkers might be more sensitive indicators of Hsp90 inhibition in vivo. We also found that the level of both proteins were elevated in sera from patients with several cancer types, suggesting that these novel secreted Hsp90-dependent proteins could be valuable pharmacodynamic tools in clinical trials of HSP90 inhibitors. Indeed, these biomarkers are being examined in ongoing multicenter phase I clinical trials of a novel formulation of 17-AAG (19).

Materials and Methods

Drugs

17-AAG and CF237 were synthesized as described previously.1 EC69 is an inactive compound and was synthesized as described (20); EC97 was synthesized as follows: adenine (47 g, 0.35 mol) was suspended in 200 mL of CHCl₃, and bromine (180 mL, 3.5 mol) was added in one portion. The suspension was stirred at room temperature for 72 hours in a closed system that was vented to a NaOH base bath. The reaction was quenched by addition of shaved ice before slowly neutralizing with aqueous ammonia to pH 8 to 9 followed by precipitation of the desired product with acetic acid. The crude product was dried under reduced pressure for 2 days to give 8-(2,5-dimethoxyphenylthio)-9H-purin-6-amine was isolated using silica gel column chromatography (CH₂Cl₂/methanol, 100:5).

Cells and Reagents

Two tumor cell lines BT474 breast cancer cells (American Type Culture Collection, Manassas, VA) and SKMG-3 glioblastoma cells (a generous gift from Chris Thomas, University of Virginia) and two normal cell lines, RPTEC human kidney epithelium (Cambrex Bioproducts) and PBMCs isolated from human blood were cultured in 10% fetal bovine serum/DMEM. Sera from normal and cancer patients were obtained from BioResearch Support (Boca Raton, FL). Antibodies used were Hsp70 (Stressgen, Ann Arbor, MI), HER-2, and phosphatidylinositol 3-kinase p85 (Upstate Biotechnology, Charlotteville, VA); all the other antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). 17-AAG and CF237 were synthesized from geldanamycin as described.1

Western Blot

Cell pellets were prepared in Western lysis buffer (10 mmol/L HEPES, 42 mmol/L KCl, 5 mmol/L MgCl₂, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L DTT, 1% Triton X-100, freshly supplemented with 1× protease inhibitor cocktail from Pierce, Rockford, IL). Lysate protein concentrations were quantified by bicinchoninic acid assay (Pierce) and normalized. Equal amounts of protein were loaded onto 4–12% Tris-glycine gels and subsequently transferred onto polyvinylidene difluoride membranes. The membranes were blocked in 5% milk in TBST. Primary antibodies were added and incubated at room temperature for 1 hour with shaking. The blots were washed extensively in TBST before secondary antibodies were added for overnight incubation at 4°C with gentle shaking. The blots were again washed extensively and developed with SuperSignal West Femto substrate (Pierce).

HER-2 Degradation by Fluorescence-Activated Cell Sorting

Cells in 24-well plates were trypsinized, washed, and pelleted as described above. Pycoerythin-labeled Anti HER-2 antibody was diluted 1:20 in BA buffer (0.2% sodium azide, 0.2% bovine serum albumin in PBS), and the cell pellets were resuspended in 100 μL of the diluted antibody. Two untreated cultures resuspended in anti-keyhole limpet hemocyanin antibody were used as background controls. Cells were incubated for 15 to 30 minutes at room temperature and were then washed twice in BA buffer and resuspended in 200 μL of the same buffer. The cell suspension was transferred to 5-mL round polystyrene tubes, an additional 300 μL of BA buffer was added, and samples were analyzed using a FACSCalibur flow cytometer equipped with Argon ion laser that emits 15 mW of 488-nm light for excitation of the pycoerythin fluorochrome.

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PBMC Isolation

Whole blood was diluted with an equal volume of sterile 1× PBS. One hundred milliliters of 54% Percoll solution was made by mixing 54 mL pure sterile Percoll, 10 mL sterile 10× PBS, and 36 mL sterile water. Fifteen milliliters of room temperature 54% Percoll solution was placed in a sterile 50-mL centrifuge tube and was overlaid carefully and slowly with 30 mL of the diluted blood with a pipette without disturbing the bottom layer. The layered blood was centrifuged at 2,000 rpm for 20 minutes at room temperature. The middle thin layer containing PBMCs was carefully removed, transferred to another sterile 50-mL centrifuge tube, and washed twice to thrice with PBS to remove Percoll.

Animal Care

Six- to 8-week-old athymic female mice were obtained from Harlan (Indianapolis, IN). The mice were maintained in sterilized ventilated caging in a room with a 12-hour light/dark cycle. Irradiated pelleted food (Harlan Teklad) and autoclaved deionized water were provided ad libitum. Animals were identified by the use of individually numbered ear tags. Experiments were carried out under institutional guidelines for the proper and humane use of animals in research established by the Institute for Laboratory Animal Research.

Tumor Model

Mice were inoculated s.c. with $10 \times 10^6$ BT474 cells supplemented with Matrigel (BD Biosciences, San Jose, CA). Mice with established tumors ($\sim 200 \text{ mm}^3$) were randomized into four groups of three for the study. 17-AAG was formulated in emulsion containing Phospholipon 90 G, Miglyol 812 N, sucrose, and water and was given i.p. at 10, 30, and 90 mg/kg. The vehicle was given for the control group. At the desired time point, serum and tumors were collected for analysis.

Tumor Processing

Snap-frozen tumors were rapidly thawed in a 37°C water bath and kept on ice. The thawed tissue was later transferred onto the lid of Petri dish, along with 50 μL of ice cold Western lysis buffer (10 mmol/L HEPES, 42 mmol/L KCl, 5 mmol/L MgCl2, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L DTT, 1% Triton X-100 and 1× protease inhibitor cocktail; Pierce). The tissue was then cut into small pieces, residual skin was removed, and the remaining tissue clumps were minced further and transferred into 250 to 500 μL of ice-cold Western lysis buffer. Sonication at setting 3 on Fisher Scientific’s Sonic Dismemberator 550 was done until no more solid was visible. The lysate was centrifuged at 12,000 rpm (14,000 × g), 4°C for 5 minutes; supernatant was collected into a fresh tube on ice; and total protein concentration (14,000/C2) was visible. The lysate was centrifuged at 12,000 rpm Dismemberator 550 was done until no more solid remaining tissue clumps were minced further and transferred onto the lid of Petri dish, along with 50 μL diluted protein solution.

IGFBP-2 ELISA

The level of IGFBP-2 was measured using an IGFBP-2 ELISA kit from R&D Systems (Minneapolis, MN) according to the manufacturer's protocol. In brief, 100 μL diluted capture antibody was coated into 96-well plates by incubation at 4°C overnight. The plate was washed and blocked with blocking buffer for 1 hour at room temperature. Samples and serially diluted standards were added into each well and incubated for 2 hours followed by further incubation with detection antibody for 2 hours. All reactions were done at room temperature, and the plate was washed thoroughly between each step. Streptavidin-horseradish peroxidase was added and left on for 20 minutes in the dark followed by a further 20-minute incubation with horseradish peroxidase substrate. The reaction was stopped by adding 50 μL stop solution to each well, and the absorbance was determined using a microplate reader set at 450 and 540 nm. The reading at 540 nm was subtracted from the readings at 450 nm to correct for optical imperfections in the plate. Statistical analyses employed the one-tailed Student’s t test.

HER-2 ELISA

The secreted extracellular domain of HER-2 in culture medium, and serum samples was measured by an ELISA kit from Calbiochem (San Diego, CA). The assay was done according to the manufacturer’s protocol. The absorbance was detected using a spectrophotometric plate reader at dual wavelengths of 450/595 nm (or 450/540 nm). Statistical analyses employed the one-tailed Student’s t test.

Results

Response of Intracellular Biomarkers to Hsp90 Inhibitors

The most commonly used biomarkers in 17-AAG clinical trials to date are Raf-1, cyclin-dependent kinase 4, and Hsp70. The first two are Hsp90 client proteins that are destabilized by Hsp90 inhibition, whereas Hsp70 is a prominent component of the HSP that is induced by Hsp90 inhibitors. In this report, we first examined the responses of these proteins to 17-AAG and CF237, a dimeric ansamycin Hsp90 inhibitor that is active against a variety of tumors. Overexpression of IGFBP-2 and HER-2 Extracellular Domain in Sera of Human Cancer Patients

Both IGFBP-2 and HER-2 extracellular domain are secreted peptides, which are detectable in human serum.
Several studies have shown that IGFBP-2 is elevated in the serum of a variety of human cancer patients, including those with melanoma or carcinoma of the adrenal cortex, ovary, and cervix (8–11). The level of IGFBP-2 correlates positively with tumor progression and spikes at relapse, thus indicating poor prognosis (22). Furthermore, reducing IGFBP-2 by either neutralizing antibody or antisense drugs significantly retarded the proliferation of prostate cancer cells, indicating that IGFBP-2 can itself play a role in tumor growth (8). As for HER-2, shedding of the extracellular domain to serum has also been recorded in several types of cancers, especially in HER-2-overexpressing breast cancer (16). High level of HER-2 extracellular domain in patient’s serum was shown to be closely related to poor outcome (16, 18).

To examine the expression of these two proteins in cancer patients’ sera, we acquired some patient serum samples from BioResearch Support. These included five of each from prostate, breast, brain cancer, and melanoma patients and five from healthy individuals. As shown in Fig. 3A, the IGFBP-2 levels were significantly elevated in breast cancer sera compared to healthy individuals.

Figure 1. Structures of Hsp90 inhibitors used in this study. 17-AAG is an ansamycin derivative. CF237 is a dimeric ansamycin containing two GM pharmacophores linked at the 17-position. EC69 and EC97 are fully synthetic Hsp90 inhibitors developed at Conforma Therapeutics.

Figure 2. Intracellular biomarkers in normal and malignant human cells. Cells were treated with DMSO or 1 μmol/L 17-AAG or CF237 for 24 h, washed, and incubated in drug-free medium for another 48 h. Expression of HSP90 client proteins was measured by Western blot, using specific antibodies against each protein. PI-3Kp85 (a nonclient protein) was used as a loading control.
(P = 0.037) and brain (P = 0.037) cancer patients and were moderately elevated in prostate and melanoma patients, respectively. In the case of HER-2 extracellular domain, the most significant elevations were seen in breast cancer patients (P = 0.0009) followed by those with prostate (P = 0.067) and brain cancers. In contrast, no HER-2 extracellular domain outside of the reference range was detected in these melanoma patients (Fig. 3B). The increased expression of IGFBP-2 and HER-2 extracellular domain in the serum of cancer patients implied that these two proteins are appropriate indicators of malignant disease.

**Response of Secreted Biomarkers to Hsp90 Inhibitors in Cell Culture**

Because the expression of both IGFBP-2 and HER-2 extracellular domain is directly or indirectly regulated by Hsp90, we tested whether Hsp90 inhibitors repressed the secretion of the two markers. To this end, BT474 cells were treated with serial dilutions of either 17-AAG or CF237. Culture medium was collected at 48 hours and examined for the presence of IGFBP-2 and HER-2 extracellular domain by ELISA. As shown in Fig. 4A and B, both proteins were suppressed in a dose-dependent manner by either of the drugs. This correlated very well with the degradation of cell surface or intracellular Hsp90 client proteins by Western blot, including Raf-1, HER-2, Akt, and Cdk4. However, it appeared that two secreted proteins were sensitive indicators of Hsp90 inhibition, as their responses could be detected at drug concentrations as low as 10 nM (Fig. 4C). Thus, 17-AAG almost completely blocked IGFBP-2 secretion at 100 nM, whereas the effects on AKT protein levels were modest under the same conditions.

To further characterize the relationship of Hsp90 inhibition and IGFBP-2 reduction, we looked at the degradation of the most sensitive Hsp90 client (HER-2) relative to inhibition of IGFBP-2 secretion by several Hsp90 inhibitors measured by indirect immunofluorescence and fluorescence-activated cell sorting analysis. These included two ansamycin derivatives, 17-AAG and CF237, and two structurally novel synthetic compounds (the active Hsp90 inhibitor EC66 and an inactive control compound EC97). The structures of all four drugs are shown in Fig. 1.

**Figure 3. Elevated levels of circulating IGFBP-2 and HER-2 extracellular domain (HER-2 ECD) in cancer patient sera.** Circulating IGFBP-2 (A) and HER-2 ECD (B) were measured in sera from cancer patients and healthy volunteers by ELISA, using kits from R&D Systems and Calbiochem, respectively. A serial dilution of either IGFBP-2 (A) or HER-2 ECD (B) protein was used to generate the standard curve. Columns, mean of five sera per group; bars, SE.

**Repression of IGFBP-2 and HER-2 Extracellular Domain in Mouse Serum Correlates with Hsp90 Client Degradation in Tumor Xenografts**

To explore whether the reduction of IGFBP-2 and HER-2 extracellular domain in mouse serum reflects the inhibition of Hsp90 in vivo, we examined the serum levels of the two proteins in BT474 tumor-bearing mice at various time points after treatment with 17-AAG and correlated that with the degradation of the corresponding Hsp90 clients in the tumors. Because the ELISAs for IGFBP-2 and HER-2 extracellular domain are specific for the human proteins (according to manufacture protocols; ref. 23), it was assumed that all circulating IGFBP-2 and HER-2 extracellular domain detected was derived from the xenografts. As shown in Fig. 6A, IGFBP-2 levels were substantially reduced within 6 hours after drug administration, recovering slightly at 24- and 48-hour time points and then diminishing further by 72 hours. Because IGFBP-2 is regulated via the IGFR → phosphatidylinositol 3-kinase → Akt pathway, we sought a correlation between down-regulation of IGFBP-2 and inhibition of Akt activity in the tumors. Indeed, Western blot of phosphorylated (i.e., active) Akt indicated that the time course of inhibition of Akt activity closely reflected circulating IGFBP-2 levels, suggesting that Akt is a major regulator of IGFBP-2 in this tumor, and that, at least in mice, circulating human IGFBP-2 has a short half-life. By contrast, levels of circulating HER-2 extracellular domain declined gradually over the course of the experiment and lagged behind the decline in tumor membrane HER-2 by ~24 hours (Fig. 6B), indicating that HER-2 extracellular domain persists in the blood for some time after its source (tumor extracellular domain) is lost. Thus, both IGFBP-2 and HER-2 extracellular domain responded to Hsp90 inhibition in vivo and mirrored the change of the corresponding Hsp90 clients in tumor cells, suggesting that the two serum proteins could potentially be used as markers for Hsp90-dependent effects on AKT and HER-2, respectively, in clinical trials.
Circulating IGFBP-2 Levels also Reflect Tumor Load

Because the sole source of human IGFBP-2 in tumor-bearing mice was from the BT474 xenotransplant, it prompted us to investigate whether IGFBP-2 levels in host serum could be used as a surrogate measure of tumor burden and the response of the tumors to treatment with Hsp90 inhibitors. To access this, we did a dose response study with 17-AAG in BT474 tumor-bearing mice. As expected, IGFBP-2 levels in mouse serum were suppressed by 17-AAG in a dose-dependent manner (Fig. 7A), in concordance with decreased tumor phosphorylated Akt (Fig. 7B and C). Furthermore, when tumors were excised and measured at the end of the experiment, the antitumor efficacy of 17-AAG closely correlated with diminution of circulating IGFBP-2 in mouse sera (Fig. 7D). However, the degree to which phosphorylated AKT and IGFBP-2 secretion were affected differed markedly (50% versus 95%). This is not entirely surprising because the Western blot samples were adjusted to a fixed protein loading concentration, thus negating any influence of tumor size on phosphorylated AKT levels. By contrast, the serum IGFBP-2 ELISA is affected by both tumor load and specific activity of IGFBP-2 secretion. It is plausible that the enhanced level of inhibition of serum IGFBP-2 relative to effects on phosphorylated AKT reflect the additional factor of tumor shrinkage in this experiment. Taken together, these data suggest that, provided the “background” plasma IGFBP-2 levels were sufficiently low, the biomarker could potentially be used to indicate antitumor activity in AKT-driven tumors in the clinic.

Discussion

As more and more Hsp90 inhibitors enter clinical development, identification of convenient and robust biomarkers becomes increasingly important. The most consistently responsive biomarker in 17-AAG clinical trials to date is Hsp70, which is clearly up-regulated in a majority of patients treated with doses of 17-AAG above the threshold of biological activity in several clinical studies. The other two biomarkers that have been used in National Cancer Institute–sponsored phase I trials, Raf-1 and Cdk-4, were only inconsistently affected, even when Hsp70 was induced in the same patients (5, 24). By necessity, intracellular Hsp70 has been measured by Western blot, which normally requires large amounts of cells from either a tumor biopsy or PBMCs, both of which are not trivial to obtain. This prompted us to look for serum biomarkers related to Hsp90 function that could be readily detected and quantified in the sera of patients with
a variety of tumor types. Here, we report the identification of two serum biomarkers, IGFBP-2 and HER-2 extracellular domain, that show promise as indicators of the biological activity of 17-AAG and other Hsp90 inhibitors in vitro and in vivo. We selected these two proteins because their expression is connected to Hsp90 regulation. IGFBP-2 synthesis is governed by IGFR! phosphatidylinositol 3-kinase! Akt pathway, which is supervised by Hsp90, and HER-2 extracellular domain is the shed form of the HER-2 receptor, one of the most sensitive Hsp90 clients. In this study, we confirmed the relationship between Hsp90 activity and biomarker secretion in vitro and in vivo.

Elevated expression of either IGFBP-2 or HER-2 extracellular domain has been observed in a variety of cancer patient sera and is positively associated with tumor progression. In ovarian cancer patients, surgical removal of the tumor reduced IGFBP-2 levels to within the reference range, but the protein surged again after clinical relapse (22). Similarly, in advanced breast cancer patients, high serum levels of HER-2 extracellular domain are associated with poor prognosis, shorter survival, and aggressive tumor growth (16, 18). The data suggest that the two secreted proteins are not only an indication of tumor presence but also are closely related to cancer progression. It is, therefore, reasonable to assume that the decrease of these secreted proteins can be used as an index for the reduction of tumor size. Indeed, in BT474 tumor-bearing mice treated with 17-AAG, IGFBP-2 levels did closely reflect relative tumor load at the end of the experiment. Notably, significant repression of IGFBP-2 occurred as early as 6 hours after 17-AAG administration, indicating that down-regulation of IGFBP-2 secretion is an early event upon Hsp90 inhibition (at least in BT474 cells) and raising the possibility that decreased IGFBP-2 levels could offer an early indication of potential clinical response.

In the time course study in tumor-bearing mice, the two biomarkers displayed distinct kinetics of response to 17-AAG treatment. IGFBP-2 had dropped almost 4-fold within 6 hours and recovered somewhat at the 24- and 48-hour time points followed by another marked decrease by 72 hours after drug administration (Fig. 6A). Akt is regulated by Hsp90 at two levels. First, a major upstream inducer of AKT activity in BT474 cells is HER-2, and Hsp90 inhibition leads to diminished AKT activity within minutes (25). Second, Akt itself is an Hsp90 client and is degraded after exposure to Hsp90 inhibitors but with markedly slower kinetics (26). It is tempting to speculate that the initial reduction of circulating IGFBP-2 was due to HER-2 degradation causing an immediate effect on Akt.
phosphorylation. However, because Akt protein levels were still not yet affected, it is possible that other signaling pathways partially reactivated it (25), resulting in a partial recovery of IGFBP-2 at 24 to 48 hours. Ultimately though, Akt itself is also degraded, perhaps leading to the further reduction in IGFBP-2 levels observed again after 72 hours. As would be predicted from this model, changes in Akt phosphorylation over time were also biphasic, mirroring that of IGFBP-2. Thus, the optimal time for measuring Hsp90 inhibitor-induced changes in circulating IGFBP2 may vary depending on the pathway(s) that influence AKT activity. In addition, the second wave of IGFBP-2 suppression may also reflect early reductions in tumor burden. In contrast, HER-2 extracellular domain levels decreased progressively from 6 to 72 hours, reflecting the rapid and persistent degradation of HER-2 induced by the Hsp90 inhibitor (Fig. 6B). We also observed that diminution in HER-2 extracellular domain levels lagged behind that of i.t. HER-2, suggesting that peak effects on this biomarker are likely to be seen at later time points after drug administration.

In addition to being readily detectable, IGFBP-2 also apparently amplified the biomarker response to Hsp90 inhibition. For example, in Fig. 6A and Fig. 7A, a 2-fold reduction in Akt activity in response to 17-AAG was translated into 4- to 10-fold decreases in IGFBP-2 levels, suggesting that IGFBP-2 might be a more sensitive marker for Hsp90 inhibition than the intracellular client proteins. Hsp90 family members play additional roles in intracellular trafficking and secretion (27); thus, suppression of secreted biomarkers may occur at several levels. Interestingly, a recent findings indicate that Hsp70 can be secreted by human cells (28) and detected in human serum (29), and that levels of anti-Hsp70 antibodies are increased in cancer patients (30), raising the possibility that elevation of circulating Hsp70 could also be used as a biomarker of Hsp90 inhibition. In this study, we have shown that both IGFBP-2 and HER-2 extracellular domain respond to Hsp90 inhibition in an analogous way to known Hsp90 clients in vitro and in xenografted mice, but in both cases the human tumor cells were the sole source of the marker proteins. Clinical studies will be required to determine whether these novel biomarkers are as informative in the context of additional sources of IGFBP-2 and HER-2 extracellular domain from nontumor sites. To address this, IGFBP-2 and HER-2 extracellular domain are currently being assessed in two multicenter phase I trials of CNF1010, a novel oil-in-water nanoemulsion of 17-AAG (19).

References


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