0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(6):2982–2988

17-Allylamino-17-Demethoxygeldanamycin Activity against Thyroid Cancer Cell Lines Correlates with Heat Shock Protein 90 Levels

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Heat shock protein 90 (Hsp90) is a molecular chaperone that stabilizes growth factor receptors and signaling molecules. Disruption of this action inhibits the MAPK and phosphatidylinositol-3 kinase cascades and can induce cancer cell death. The goal of this study was to determine whether thyroid cancer cells are sensitive to the cytotoxic effects of 17-allylamino-17-demethoxygeldanamycin (17-AAG), an Hsp90 inhibitor in clinical trials, and to determine predictors of this response. Papillary (NPA), follicular (WRO), and anaplastic (ARO) thyroid cancers were incubated with 17-AAG *in vitro*. Surprisingly, the ARO cells were most sensitive to the cytotoxic effects of this agent. Conversely, all cell lines displayed similar responses to specific blockers of phosphatidylinositol-3 kinase and MAPK kinase (LY294002 and U0126, respectively). Western blot demonstrated that the NPA cells that were most resistant to 17AAG-induced cytotoxicity had the lowest levels of Hsp90 and were the only cells with persistent levels of Akt protein. Interestingly, even the WRO and ARO cell lines that were sensitive to 17-AAG-induced cell death did not undergo apoptosis. These data suggest that sensitivity of thyroid cancer cells to 17-AAG-induced cytotoxicity relates to Hsp90 levels rather than histological subtype and that thyroid cancer cells have a reduced apoptotic response to 17-AAG. (*J Clin Endocrinol Metab* 89: 2982–2988, 2004)

THYROID CANCER CELL growth is regulated both by TSH and tyrosine kinase-mediated signaling pathways. For most aggressive thyroid cancers, the effects of TSH-mediated tumor growth are limited by down-regulation or the absence of TSH receptor expression (1). In addition, activating mutations of the TSH receptor are rarely found in malignant thyroid tumors, whereas overexpression, mutations, or genetic rearrangements that activate tyrosine kinase pathways are common (2). Thus, it is likely that activation of tyrosine kinase receptors is critical to thyroid cancer cell growth and progression.

Tyrosine kinase receptors activate several pathways that regulate thyroid cell growth, cell cycle progression, and cell death (3). Specifically, important roles for both the MAPK and phosphatidylinositol-3 (PI3) kinase pathways have been described in thyroid cancer cell models, *in vivo*, and in human tissue samples (4–9). Indeed, overexpression of tyrosine kinase receptors, genetic rearrangements that result in constitutive activation of tyrosine kinase receptors, mutations in genes encoding signaling molecules such as B-Raf and Ras, and inactivation of tumor suppressor genes, such as phosphatase and tensin homolog deleted on chromosome 10 (PTEN), all result in activation of tyrosine-kinase activated pathways and together account for the majority of human thyroid cancers (2). The high frequency of pathway activation in thyroid cancer, therefore, makes signaling inhibitors attractive for targeted therapy for this disease.

Akt and Raf are serine-threonine kinases that are important intermediaries in the PI3 kinase and MAPK pathways, respectively. For proper function and stabilization, these two proteins (and others) bind to heat shock protein 90 (Hsp90), a molecular chaperone that is responsible for ensuring an adequate folding of newly synthesized proteins and refolding of mature proteins undergoing conditions of denaturing stress (10). Disruption of Hsp90 binding leads to ubiquitination and subsequent proteosomal degradation of Hsp90-chaperoned proteins (11). Consistent with this mode of action, inhibition of Hsp90 leads to Raf-1 depletion and consequent inhibition of MAPK kinase (MEK)1/2 phosphorylation (12) and to degradation of Akt (13), causing reduced cell signaling, cell growth, and ultimately, cell death.

Because there is significant overactivation of these pathways in many cancer cells, Hsp90 inhibitors have been developed for use in clinical trials. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is the first Hsp90 inhibitor to enter clinical studies. It is derived from the benzoquinoid ansamycin antibiotic geldanamycin, an agent with potent antitumor activity, but also with hepatic toxicity (14, 15). 17-AAG maintains similar antitumor properties but has fewer associated side effects. It has been demonstrated to inhibit proliferation of several tumor cell lines *in vitro*, in xenograft models, and has demonstrated benefit in phase 1 and 2 clinical trials (15). 17-AAG binds the ATP-binding pocket in the amino terminus of Hsp90, thereby inhibiting

Abbreviations: 17-AAG; 17-Allylamino-17-demethoxygeldanamycin; DMSO, dimethylsulfoxide; EGF, epidermal growth factor; GI, growth inhibition; GI_{50}, GI of 50%; Hsp90, heat shock protein 90; MEK, MAPK kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide; PARP; poly-ADP-ribose polymerase; PI3, phosphatidylinositol-3.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Hsp90 function. In cancer cells, but not in normal cells, Hsp90 is part of a multichaperone complex with enhanced activity and high affinity for 17-AAG (~100-fold greater than normal cells), resulting in tumor-specific activity (16, 17). However, differences in Hsp90 activity between different cancer cell lines have not yet been thoroughly addressed.

In thyroid cancer, recent evidence has demonstrated that geldanamycin induces follicular thyroid cancer cell apoptosis, down-regulates epidermal growth factor (EGF) receptor expression, and disrupts Raf signaling, resulting in *in vitro* and *in vivo* activity, suggesting this class of agents might be active against thyroid cancer (18). Because of the frequency of abnormalities in signaling pathways that are dependent on proteins that interact with Hsp90 in thyroid cancer we sought to evaluate the effect of 17-AAG on thyroid cancer cell lines and to determine whether Hsp90 levels could serve as a predictor of response to this agent. We demonstrate that 17-AAG is active against thyroid cancer cell lines *in vitro*, that this activity correlates to endogenous levels of Hsp90 in the cancer cells rather than degree of differentiation, and that 17-AAG induces thyroid cancer cell death without inducing apoptosis.

Materials and Methods

Cell culture

Human thyroid cancer cell lines, ARO (anaplastic), NPA (papillary), and WRO (follicular carcinoma) were kindly provided from Dr. R. Julliard (University of California, Los Angeles, Los Angeles, CA). Cells were maintained in RPMI 1640culture medium supplemented with 5% fetal bovine serum and 1× nonessential amino acids (1%) (all purchased from Invitrogen Corp., Carlsbad, CA) at 37 C and humidified 5% CO_2. Cells were washed in PBS and placed in RPMI 1640 medium in 12-well plates for 24 h before experiments. All inhibitors were diluted in dimethylsulfoxide (DMSO) per the manufacturer's recommendations, and control experiments adding equivalent concentrations of DMSO in the absence of inhibitors were performed for each experiment. Pharmacological inhibitors of PI3 kinase and MEK (LY294002 and PD98059, respectively) were purchased from Cell Signaling Technology (Beverly, MA). 17-AAG was kindly supplied by Dr. A. Fallavollita (Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD) and was also dissolved in DMSO.

Protein extraction. Cells were placed in 10-cm dishes and cultured until 50% confluent. After washing with PBS, cells were cultured in fresh serum-free medium for 24 h, and experiments were performed with blockers at the concentrations and time points noted. To stop the experiments, cells were rinsed twice with 1 ml of ice-cold PBS, 0.5 ml of ice-cold cell lysis buffer [20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4 (Sigma, St. Louis, MO), 1% Triton X-100 (Sigma), 20 μM phenyl methyl sulforyl fluoride (Roche Molecular Biochemicals, Indianapolis, IN) and 0.3 μM okadaic acid (Sigma)] was added, and the dish was incubated on ice for 10 min. The cells were scraped and centrifuged at 12,000 × *g* for 10 min at 4 C. The supernatant was saved and stored at −80 C. Protein content of the supernatant was quantified using bicinchoninic acid kit (Pierce Biotechnology, Inc., Rockford, IL).

Immunoblotting and protein detection. Antitotal Akt, phospho-Akt (Ser 473 and Thr 308), ERK, phospho-ERK, (Cell Signaling Technology), and anti-poly-ADP-ribose polymerase (PARP) (Roche Diagnostics, Indianapolis, IN) antibodies were used as primary antibodies. For Western blots, 20 μg of total protein lysate was suspended in reduced sodium dodecyl sulfate sample buffer and boiled for 5 min. Protein lysates were subjected to SDS-PAGE (8%), and the separated proteins were transferred to nitrocellulose membranes (0.45 μm pore size, Invitrogen) by electrophoretic blotting (Invitrogen). Nonspecific binding was prevented by blocking the membrane with 0.1% Tween 20 in PBS (PBS-T) containing 5% nonfat dry milk overnight at 4 C. Immunoblotting was performed in the following manner: in brief, membranes were washed four times (10 min/wash) with PBS-T, incubated with the primary antibody in PBS-T containing 5% BSA or nonfat dry milk for 2 h at room temperature and washed four times with PBS-T (15 min/wash). Membranes were then incubated with the secondary antibody conjugated with peroxidase (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in PBS-T containing 5% nonfat dry milk for 1 h at room temperature. After washing with PBS-T four times (15 min/wash), immunodetection was performed by using SuperSignal West Pico staining kit (Pierce).

Cell growth studies

Cell survival and proliferation was determined by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) (Sigma)] assay. Cells were plated in 12-well dishes and grown until 30% confluence was reached, after which medium was replaced by 17-AAG diluted in culture medium in concentrations ranging from 1 nM to 100 μM for up to 96 h. At 72 h, the original medium was replaced by fresh medium with 17-AAG diluted in the same concentrations. Each experiment was performed three times in triplicate. One hundred microliters of 5 mg/ml MTT assay was added to each well, and cells were subsequently returned to the incubator for 4 h. Isopropanol with 0.04 N HCl (1 ml) was added, and absorbance on a 96-well plate reader with a wavelength of 570 nM was measured in a 150-μl sample (Revelation 4.02, DYNEX Technology, Chantilly, VA).

To assess total DNA content, cells were seeded in 12-well plates at 10^5 cells per well and incubated for 24 h. 17-AAG in similar concentrations as described above was added to each well for 24, 48, 72, and 96 h. After incubation, cells were washed twice with ice-cold PBS (Life Technologies, Gaithersburg, MD), and the amount of DNA in the cells was measured with diphenylamine reagent after perchloroacetic acid precipitation.

Statistical analysis. Measurements of DNA content and MTT assays were repeated at least three times in triplicate. Values are the mean ± SD of these experiments. All Western blot experiments were repeated on at least three separate occasions to confirm results. Significance between values was determined by ANOVA and *post hoc* Fisher Test (significant if $P < 0.05$) using STATVIEW (Abacus Concepts, Inc., Berkeley, CA).

Results

17-AAG inhibits growth of thyroid cancer cells

ARO, NPA, and WRO human thyroid cancer cells were grown in 12-well plates and treated with 17-AAG in increasing concentrations for 24, 48, and 72 h at concentrations ranging from 0.1–10 μM. As determined by DNA content, the number of living cells was reduced in the presence of 17-AAG for all cell lines (Fig. 1, $P \leq 0.001$). Interestingly, only ARO cells, the line derived from the most aggressive thyroid cancer, developed an apparent cell death response in which the 17-AAG treatment resulted in cell numbers lower than the initial d 0 cell number at both 48 and 72 h (Fig. 1). The concentrations of 17-AAG able to attain growth inhibition (GI) of 50% (GI_{50}) and 100% (total GI) are demonstrated in Table 1. Similar results were obtained using MTT assays (data not shown).

Sensitivity to 17-AAG is different from direct signaling inhibition

Because of the surprising sensitivity of the ARO cells to 17-AAG in comparison with that of the other cell lines and the relative resistance of the NPA cell line, we sought to define whether or not this difference was due to the signaling inhibitory properties of 17-AAG or to other Hsp90-dependent effects. Thus, we compared the activity of 17-AAG with

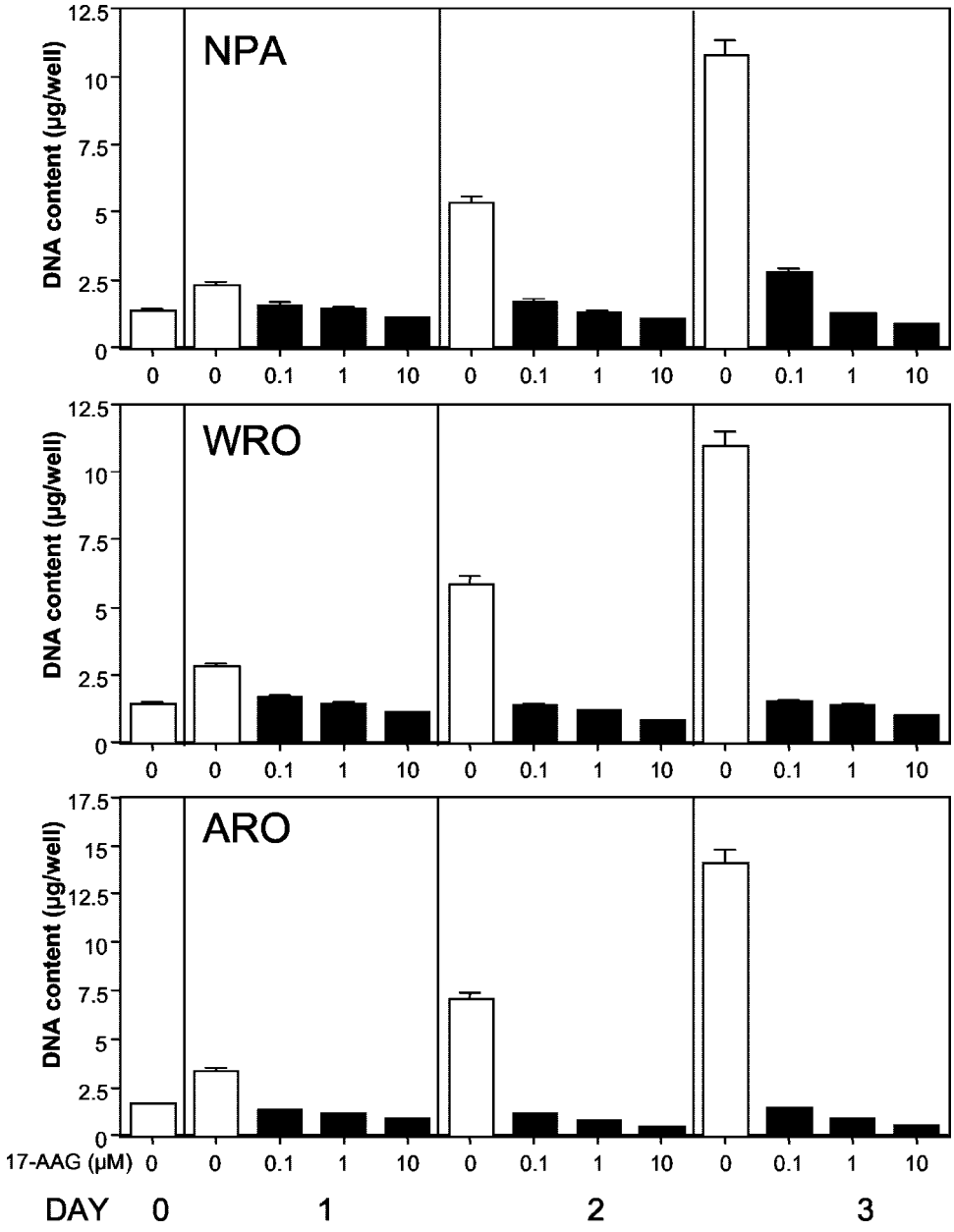

FIG. 1. Dose and time response of thyroid cancer cell lines to 17-AAG. NPA, WRO, and ARO cells were incubated with DMSO (0, *white bars*) or increasing concentrations of 17-AAG for 1, 2, and 3 d in the absence of serum. DNA content increased in control cells, indicating cell growth of all lines without treatment. A concentration of 0.1 mM was partially growth inhibitory in NPA cells, completely growth inhibitory in WRO cells, and induced cell death (cell number below d 0 control) in ARO cells. Results are the mean values from three separate experiments performed in triplicate. GI was statistically significantly lower at all doses and at all time points compared with control cell growth ($P = 0.001$ for 0.1 μM for NPA, all others <0.001)

TABLE 1. 17-AAG concentrations needed to attain GI_{50} and total GI

Cell line	Inhibitor		
	AAG (μM)	LY (μM)	U (μM)
GI_{50}			
NPA	0.1	11	8.1
WRO	0.04	7.2	14
ARO	0.03	6.3	18
Total GI			
NPA	9	52	26
WRO	0.2	62	90
ARO	0.16	64	140

LY, LY294002; U, U0126.

those of direct inhibitors of the PI3 kinase and MAPK pathways using specific inhibitors of PI3 kinase (LY294002) and MEK (U0126) at low doses. After 72 h of incubation, the ARO and WRO cell lines were more sensitive than NPA cells to 17-AAG (Fig. 2A). This result was distinct from the direct PI3 kinase and ERK inhibitors (Fig. 2, B and C), and the three cell lines were similarly sensitive to the combination of LY294002 and U0126 (data not shown). Taken together, these data confirmed that the NPA cell line was less sensitive than other cell lines to 17-AAG and that this was specific for this particular agent, suggesting that the degree of signaling pathway activity would not predict the biological activity of 17-AAG in these cell lines.

Sensitivity to 17-AAG is correlated with Hsp90 levels and Akt stability

To determine whether the degree of degradation of Hsp90 targets and/or cell signaling effects correlated with 17-AAG response in thyroid cancer cell lines, we evaluated

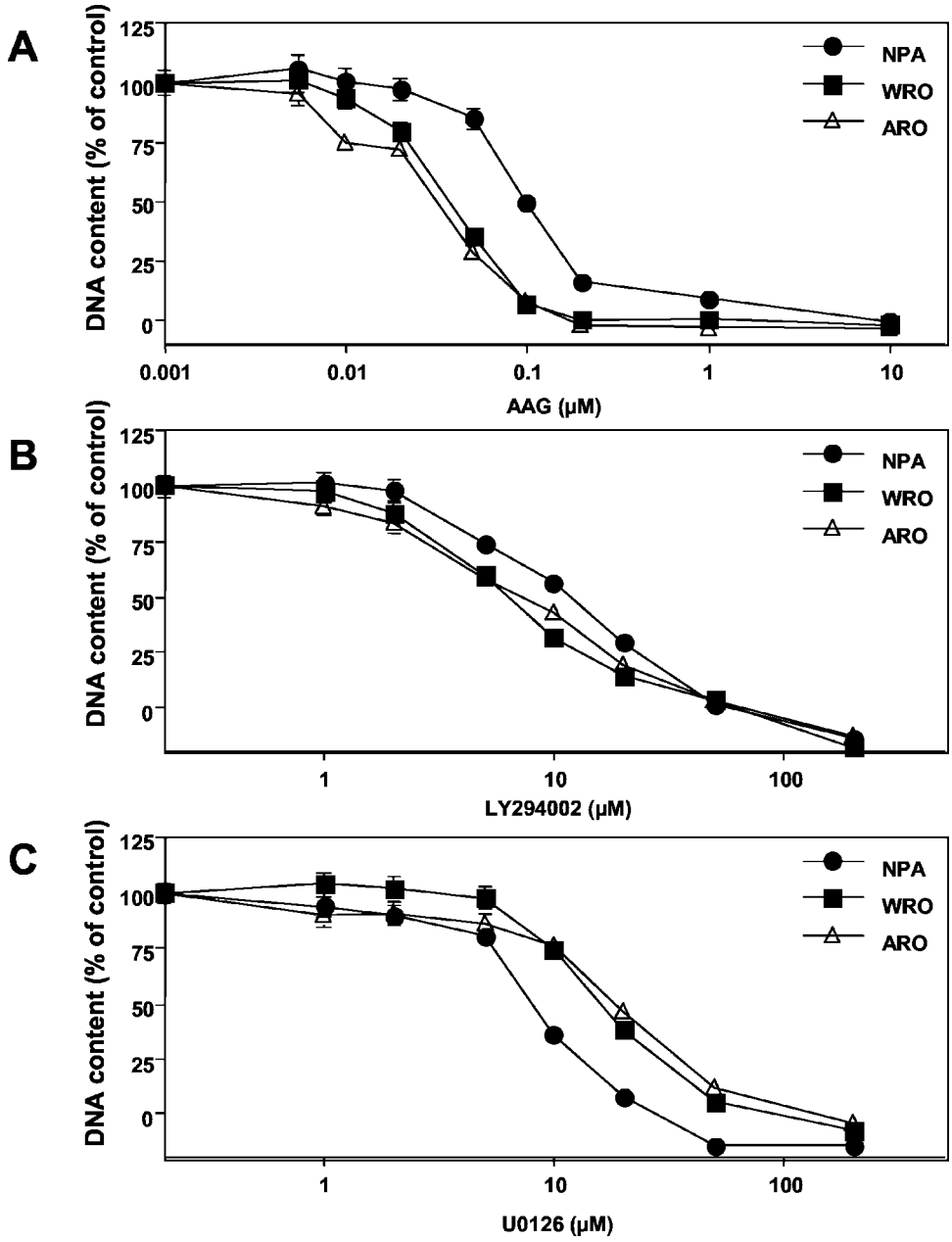

FIG. 2. Comparison between 17-AAG and specific inhibitors of PI3 kinase and MEK on thyroid cancer cell lines. NPA, WRO, and ARO cells were incubated with 17-AAG (Hsp90 inhibitor) (A), LY294002 (PI3 kinase inhibitor) (B), or U0126 (MEK inhibitor) (C) for 72 h. DNA content was measured and compared with DMSO control and dose-response curves were plotted. The relative resistance of NPA cells was unique to treatment with the Hsp90 inhibitor and was not demonstrated with either signal transduction inhibitor. Results are the mean values with SEs for three separate experiments performed in triplicate.

the effects of 17-AAG, LY294002, and U0126 on Hsp90 expression, levels of total and phosphorylated (active) Akt, and total and phosphorylated (activated) ERK1/2 by Western blot analysis (Fig. 3). Control experiments with vehicle alone were included for all cell lines, and they demonstrated that Hsp90 levels were lower in NPA cells than in WRO and ARO cells. Akt and ERK were activated in all three cell lines (control lanes) at 72 h. Disruption of Hsp90 binding would be expected to cause increased degradation of Akt leading to reduced levels of total and activated Akt. Because Hsp90 does not bind directly to ERK, total ERK levels are expected to be stable, but phosphorylated ERK should be reduced due to enhanced degradation of its upstream activator, Raf-1, creating a pattern that is a "bioassay" for reduced Raf-1 stability and activity. This pattern is demonstrated for WRO and ARO after 72 h of 17-AAG treatment. In comparison, NPA cells demonstrated similar blockade of ERK activation but retained detectable levels of total Akt after 72 h of treatment, even using higher doses of 17-AAG (10 μM). Although Akt activity was inhibited after 72 h of exposure, the retention of Akt levels is consistent with reduced effects of Hsp90 inhibition on cell number (Figs. 1 and 2) and with the lower levels of Hsp90 protein. Conversely, the specific inhibitory effects of LY294002 and U0126 were similar between the three cell lines.

The dose and time relationships of this response were more carefully evaluated (Fig. 4) and demonstrated that at lower doses, levels of total Akt and Akt activation were maintained to a greater degree in NPA cells compared with WRO and ARO cells. In addition, despite similar reductions of cell number induced by 1 μM 17-AAG for 72 h, there was

a greater level of escape from the Akt inhibitor after 96 h in these cells, consistent with relative resistance to 17-AAG.

17-AAG-induced cytotoxicity does not correlate with apoptosis of thyroid cancer cell lines

Because 17-AAG has been shown to induce apoptosis of cancer cell lines, and thyroid cancer cells are frequently resistant to apoptosis, we evaluated whether or not this agent was able to induce apoptosis in sensitive cell lines. For these experiments, two measures of apoptosis were assessed: cleavage of PARP (19) and DNA laddering. PARP cleavage was not identified in any of the thyroid cancer cell lines after exposure to even high doses of 17-AAG (Fig. 5A). By contrast, human melanoma cell lines displayed an apoptotic response to 17-AAG (Fig. 5B). These negative results were confirmed in DNA laddering experiments (data not shown).

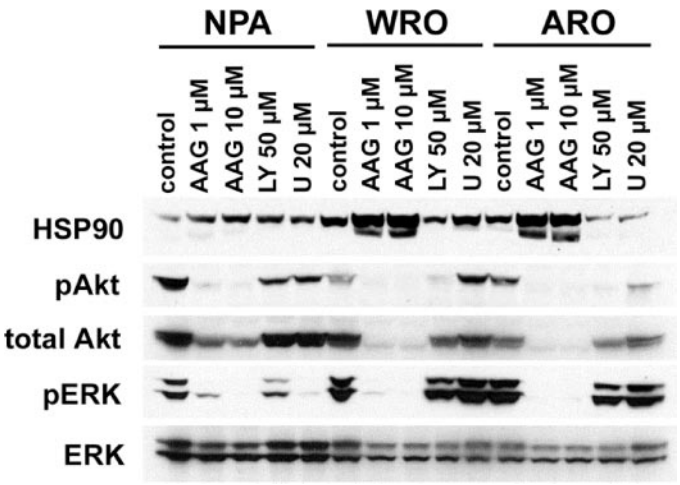

FIG. 3. Inhibition of cell signaling by Hsp90, PI3 kinase, and MEK inhibitors. To further compare the effects of 17-AAG (AAG), LY294002 (LY), and U0126 (U) on thyroid cancer cell lines, Western blots for Hsp90, phosphorylated Akt (pAkt), total Akt, phosphorylated ERK (pERK), and total ERK were performed after 72 h of exposure. Hsp90 levels were lower in NPA cells than in WRO and ARO cells. As expected, LY blocked Akt phosphorylation without altering total Akt levels, and U blocked ERK phosphorylation without altering levels of total ERK consistent with pathway-specific blockade. 17-AAG treatment resulted in loss of total Akt and active Akt in all three cell lines, although this effect was less dramatic for total Akt in the NPA cells. In addition, 17-AAG blocked ERK activation but did not cause degradation of total ERK, as expected because ERK is not a direct target of 17-AAG (the upstream regulator of ERK, Raf-1, is a client protein of Hsp90). These results are representative of three separate experiments.

Discussion

Patients with progressive and metastatic thyroid cancer have a 10-yr mortality rate of approximately 50% and do not typically respond to chemotherapeutic agents (20). The pathways responsible for the continued survival and growth of these tumors are being elucidated and include the growth factor receptor-coupled MAPK and PI3 kinase signaling cascades. Agents that target components of these pathways, from receptor blockers to small molecule inhibitors of signaling proteins, are in development, and some may have activity against thyroid cancer (21, 22). Mechanistically, inhibitors of Hsp90 are an attractive family of agents for thyroid cancer because they will inhibit both PI3 kinase and MAPK and induce down-regulation of growth factor receptors simultaneously.

Hsp90 has two isoforms (23) and plays an important role in the stabilization of protein conformation and nuclear

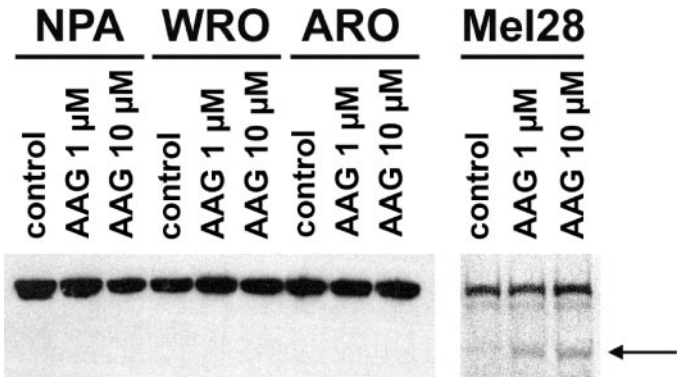

FIG. 5. 17-AAG does not induce apoptosis in thyroid cancer cell lines. To determine whether 17-AAG induced apoptosis of thyroid cancer cell lines, NPA, WRO, and ARO cells (*left panel*) and human melanoma cells (SK-MEL 28, American Type Culture Collection) were treated with 17-AAG for 72 h at doses that cause cell death or are growth inhibitory (Figs. 1 and 2), and cleavage of PARP was assessed by Western blot. The thyroid cancer cell lines displayed only full-length PARP after 72 h of exposure, whereas the melanoma cells display PARP cleavage as evidenced by the emergence of a second, more rapidly migrating band (*arrow*). These results are representative of four separate experiments.

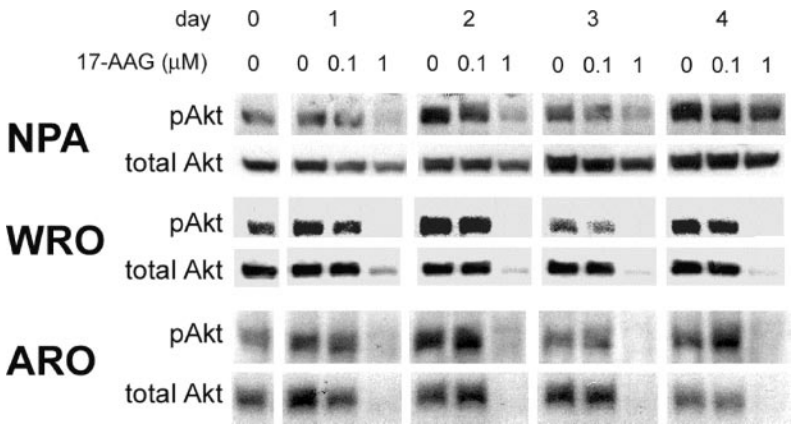

FIG. 4. NPA cells are relatively resistant to 17-AAG. To further compare the direct effects of 17-AAG on thyroid cancer cell lines, cells were treated with a lower dose (0.1 and 1 mM) or DMSO for 96 h, and Western blots were performed after each 24 h of exposure for total and phosphorylated Akt. In comparison with the WRO and ARO cell lines, total Akt levels were preserved in NPA cells, and the escape from the effects of the agent occurred at lower concentrations after 96 h of exposure. These results are representative of three separate experiments.

translocation of a wide variety of proteins, including Raf-1, Akt, tyrosine kinase receptors (including the EGF receptor and c-Met, steroid family receptors, and cyclin-dependent kinase 4 (10, 24). Disruption of Hsp90 binding to client proteins with 17-AAG has been shown to deplete cancer cells of Raf 1 and Akt, leading to reduced activation of Akt and ERK *in vitro* and *in vivo* (12, 13, 25). More recently, a mechanism that accounts for the tumor-specific effects of 17-AAG in comparison with nonmalignant cells has been reported. In cancer cells, Hsp90 is bound to a chaperone complex that is more active in binding affinity for client proteins and also for binding 17-AAG. In comparison, nonmalignant cells are characterized by free, noncomplexed latent Hsp90 (16, 17). Finally, Hsp90 can be modified by phosphorylation, resulting in enhanced activity and increased responsiveness to geldanamycin (26). Taken together, the evidence suggests that this class of agents has potential to produce tumor-targeted signal transduction inhibition.

Recent studies using the parent compound of 17-AAG, geldanamycin, have documented activity against follicular thyroid cancer cell lines *in vitro* (18). In this study, the follicular cancer cell line FTC 133 was more sensitive to geldanamycin than ARO. Apoptosis was detected, but only with high doses of geldanamycin and after 96 h of incubation using a highly sensitive annexin V assay. Reduced expression of Raf-1 and EGF receptor were noted in the FTC 133 cell line, but no comparisons between the more- and less-sensitive cell lines were reported. It is important to recognize that different geldanamycin derivatives, including 17-AAG and the more water-soluble 17-DMAG, have been shown to induce cell death with different efficiencies in the same cell lines (15). The mechanisms for differences between these closely related agents are uncertain but may reflect the maintenance of intracellular concentrations of the agent or activation by enzymes, such as reduced nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1 (also known as DT-diaphorase), a quinine reductase that activates 17AAG but not geldanamycin (27). Importantly, geldanamycin is not likely to be clinically useful due to hepatotoxicity in animal studies, whereas 17-AAG and 17 DMAG are both in clinical trials (15).

In the present study, we evaluated the sensitivities of several different thyroid cancer cell lines to 17-AAG at concentrations similar to or below the GI_{50} and 50% lethal concentration for these agents in humans. Because benign human thyroid cell lines are not readily available, we examined the effect of 17-AAG on the cell proliferation of the benign rat thyroid cell line, FRTL-5. GI_{50} for these cells was approximately 1 μM (data not shown), approximately 30-fold higher than the ARO and WRO lines, and 10-fold higher than the NPA cell line. The GI_{50} and total GI concentrations for the ARO and WRO cell lines are similar to those noted for cell lines derived from cancers that have been sensitive to 17-AAG *in vivo* (15) and are lower than those achieved in the serum of patients in phase 1 studies (28). In contrast to geldanamycin, ARO cells were more sensitive to 17-AAG than concomitantly tested follicular and papillary cancer-derived cell lines. This sensitivity did not appear to correlate directly with activation of Akt or ERK in that this differential result was not apparent using direct inhibitors of these pathways, LY294002 and U0126, respectively. However, the degree of sensitivity did correlate with levels of Hsp90 protein expression and with loss of Akt protein, consistent with the mechanism of action of the agent.

We were surprised that the ARO cell line, the line derived from the tumor type that is typically more resistant to most agents, appeared to be more sensitive to 17-AAG. This suggests that the levels of the direct target of the drug, Hsp90, might predict biological activity of 17-AAG better than the histological tumor type. Alternatively, enhanced bioactive, phosphorylated, or complexed Hsp90 might be another predictor of 17-AAG activity. In either case, determination of tumoral levels of total and/or bioactive Hsp90 might predict which tumors will respond to this class of drugs, thereby providing an opportunity to tailor targeted therapy for particular tumors.

Also in contrast to the prior study of Park *et al.* (18), the thyroid cancer cell lines did not undergo apoptosis in response to 17-AAG. It is possible that 17-AAG induced necrotic cell death, that the degree of apoptosis was below the sensitivity of PARP cleavage or DNA laddering methods, or that a longer time of exposure to higher doses of 17-AAG is needed to induce apoptosis. In any case, it is likely that nonapoptotic mechanisms primarily account for the majority of the reduction of cell viability induced by 17-AAG treatment because there was no evidence of PARP cleavage or DNA laddering induced by the agent over 72 h of exposure. The reduced apoptotic response to 17-AAG for thyroid cancer cells in comparison with melanoma is consistent with prior data demonstrating that many benign and malignant thyroid cells display resistance to apoptosis. It is possible that mutations of the p53 gene play a role in the resistance to 17-AAG-induced apoptosis, because all three lines are characterized by p53 mutations; however, it is not likely that they are responsible for their variable cytotoxic response. The mechanism for resistance to 17-AAG-induced apoptosis is uncertain, but reduced sensitivity to Fas- and TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis are well described and characterized (29, 30).

In summary, we have demonstrated that thyroid cancer cell lines are sensitive to the Hsp90 inhibitor 17-AAG. The sensitivity to the cytotoxic effects of this agent correlated with the level of Hsp90 expressed in the tumor cell line and correlated with the loss of a direct Hsp90 target, Akt. This effect appeared to be distinct from just the inhibition of cell signaling and likely relates to disruption of Hsp90 binding to other client proteins in addition to Akt and Raf-1. Therefore, it appears that 17-AAG is an agent with activity against thyroid cancer cell lines *in vitro* and that this activity can be predicted by the level of Hsp90 in the cell lines.

Acknowledgments

We thank Richard T. Kloos, M.D., for his constructive review of this manuscript.

Received October 9, 2003. Accepted November 13, 2003.

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This work was supported, in part, by The American Cancer Society (Grant RSG CNE1888-02 to M.D.R.) and the National Cancer Institute (Grant CAN8339479 to M.D.R.).

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