Antitumor Activity of SNX-2112, a Synthetic Heat Shock Protein-90 Inhibitor, in MET-Amplified Tumor Cells with or without Resistance to Selective MET Inhibition

Thomas Bachleitner-Hofmann, Mark Y. Sun, Chin-Tung Chen, David Liska, Zhaoshi Zeng, Agnes Viale, Adam B. Olshen, Martina Wittboeck, James G. Christensen, Neal Rosen, David B. Sloit, and Martin R. Weiser

Abstract

**Purpose:** Heat shock protein-90 (HSP-90), a molecular chaperone required by numerous oncogenic kinases [e.g., HER-2, epidermal growth factor receptor (EGFR), Raf-1, v-Src, and AKT] for conformational stability, has attracted wide interest as a novel target for cancer therapy. HSP-90 inhibition induces degradation of HSP-90 client proteins, leading to a combinatorial inhibition of multiple oncogenic signaling pathways with consecutive growth arrest and apoptosis. MET, a tyrosine kinase that is constitutively active in tumor cells with MET oncogene amplification, has recently been identified as another HSP-90 client.

**Experimental Design:** The aim of our study was to assess the efficacy of SNX-2112, a synthetic HSP-90 inhibitor, in 3 different MET-amplified tumor cell lines (GTL-16, MKN-45, and EBC-1) as well as PR-GTL-16 cells, a GTL-16 subline selected for resistance to the highly selective MET kinase inhibitor PHA-665752.

**Results:** In all cell lines, SNX-2112 led to degradation of MET, HER-2, EGFR, and AKT, as well as abrogation of Ras/RAF/MEK/MAPK and PI3K/AKT signaling, followed by complete cell cycle arrest. SNX-5542, an orally bioavailable prodrug of SNX-2112, displayed significant antitumor efficacy in mice bearing MET-amplified tumor xenografts. Importantly, HSP-90 inhibition maintained its antitumor efficacy in PR-GTL-16 cells both in vitro and in vivo, suggesting that HSP-90 inhibition could be a particularly valuable strategy in MET-amplified tumors that have acquired resistance to MET kinase inhibition.

**Conclusions:** Our study provides evidence for the efficacy of HSP-90 inhibition in MET-amplified cancer cells, particularly when MET kinase inhibitor resistance has emerged. Clin Cancer Res; 17(1); 122–33. ©2011 AACR.
cells either through MET-dependent downstream signaling or receptor cross talk (e.g., Raf, AKT, and EGFR, refs. 5–7, 19). Furthermore, MET itself has recently been implicated as an HSP-90 client (20–24). We therefore hypothesized that HSP-90 inhibition could be a particularly promising treatment strategy in MET-amplified cancer cells. Moreover, due to its combined effect on multiple signal transduction pathways, we hypothesized that HSP-90 inhibition could also overcome acquired resistance to small molecule MET inhibition in these malignancies. In this study, we have tested the effects of SNX-2112, a novel synthetic HSP-90 inhibitor (25–27), in 3 different tumor cell lines with MET amplification [EBC-1 (non–small-cell lung cancer), GTL-16 (gastric cancer), and MKN-45 (gastric cancer)] as well as PHA-resistant (PR)-GTL-16 cells that we selected for acquired resistance to PHA-665752, a highly selective MET kinase inhibitor. In all cells, degradation of MET was observed together with degradation of the HSP-90 clients HER-2, EGFR, and AKT. MET degradation was paralleled by loss of MET phosphorylation, abrogation of downstream PI3K/AKT, and Ras/Raf/MEK/MAPK signaling as well as by cell cycle arrest. HSP-90 inhibition using SNX-5542, an orally bioavailable prodrug of SNX-2112, also displayed significant antitumor activity in vivo in nude mice bearing MET-amplified xenografts with minimal toxicity. Importantly, HSP-90 inhibition maintained its in vitro and in vivo antitumor efficacy in PR-GTL-16 tumor cells with acquired resistance to PHA-665752, providing a strong rationale for the use of HSP-90 inhibition in MET-amplified tumors that have become resistant to selective MET kinase inhibition.

Materials and Methods

Cell lines
Human GTL-16 gastric cancer cells were a gift from Dr. Silvia Giordano (Institute for Cancer Research and Treatment, Torino School of Medicine, Italy). MKN-45 gastric cancer cells were obtained from the RIKEN BRC Cell Bank (RIKEN BioResource Center). EBC-1 non–small-cell lung cancer cells were from the Health Science Research Resources Bank (Japan Health Sciences Foundation). NCI-H820 cells were obtained from the American Type Culture Collection. GTL-16 and PR-GTL-16 cells were grown in Dulbecco’s modified Eagle’s Medium, MKN-45 and NCI-H820 cells were grown in RPMI-1640, and EBC-1 cells were grown in Eagle’s minimal essential medium + 2 mmol/L L-glutamine + 1 mmol/L sodium pyruvate + 0.1 mmol/L nonessential amino acids. All media were supplemented with 10% fetal calf serum and maintained at 37°C in a humidified atmosphere containing 5% CO2.

Chemicals
PHA-665752, PD173074, and PD330631 were provided by Pfizer Global Research and Development. Gefitinib (ZD-1839; Iressa) was obtained from AstraZeneca Pharmaceuticals. Recombinant human fibroblast growth factor-3 (FGF-3) was purchased from R&D Systems. SNX-2112 (for chemical structure see Supplementary Fig. S1) and SNX-5542 were obtained from Serenex, Inc. SNX-2112 was dissolved in dimethyl sulfoxide (DMSO) for in vitro studies, whereas SNX-5542 was formulated in 5% dextrose in water for in vivo studies.

Western blot
After removal of growth medium, tissue culture flasks were placed on ice and washed twice with ice-cold Tris-buffered saline (TBS). Cells were scraped off the culture flasks, centrifuged, and placed in ice-cold cell lysis buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors (Halt protease and phosphatase inhibitor cocktails; Pierce). After shaking for 15 minutes at 4°C, the lysates were centrifuged at 20,000 × g for 15 minutes and stored at −70°C until further use. For Western blotting, equal amounts of protein (50 μg) were boiled in Laemmli buffer for 5 minutes, resolved by 10% SDS-polyacrylamide gel electrophoresis (Invitrogen) and electrophoretically transferred onto a polyvinylidene difluoride membrane (BioRad). After blocking nonspecific binding sites with 5% nonfat dry milk in TBS + 0.05% Tween 20 (TBS-T), the membrane was incubated with the respective primary antibodies. After 3 washes with TBS-T, the membrane was incubated for 1 hour at room temperature with a horseradish peroxidase-linked secondary antibody, followed by several washes with TBS-T. The immunocomplexes were visualized using the ECL Plus detection system (GE Healthcare).

Antibodies
Antibodies against MET (C-12), EGFR (1005), and cyclin D1 (M-20) were from Santa Cruz Biotechnology.
Antibodies against phospho-MET (Tyr1230/1234/1235), p44/42 MAPK, phospho-p44/42 MAPK (Thr202/Tyr204), AKT, and phospho-AKT (Ser473) were from Cell Signaling Technology. Anti–HER-2 antibody (clone e2-4001) was from Lab Vision.

Cell proliferation assays

Cellular proliferation was measured using a commercially available 5-bromo-2-deoxyuridine (BrdU) cell proliferation assay (Roche). Briefly, the cells were seeded in triplicate in flat-bottom 96-well plates at 5,000 cells per well and allowed to adhere for 48 hours. Thereafter, the cells were treated for 24 hours, as indicated. After incubation with BrdU labeling reagent for 2 hours, the cells were fixed and BrdU incorporation into newly synthesized DNA was assessed by incubation with an anti-BrdU peroxidase-conjugated antibody for 90 minutes, followed by addition of substrate solution and colorimetric detection at 450 and 690 nm, respectively. IC_{50} values were calculated by 4-parameter curve fitting using SigmaPlot 11.0 software.

Apoptosis

Induction of apoptosis was assessed using the Annexin V FITC Apoptosis Detection Kit from BD Pharmingen (BD Biosciences). Briefly, the cells were treated with SNX-2112 for 24 hours, stained with Annexin V FITC + propidium iodide (PI) at room temperature according to the manufacturer’s instructions, and finally subjected to flow cytometric analysis.

RNA isolation, labeling, and microarray analysis

Total RNA was isolated from tissue using the RNeasy Kit (Qiagen). The quality of the RNA was ensured before labeling by analyzing 20 to 50 ng of each sample using the RNA 6000 NanoAssay and the Bioanalyzer 2100 (Agilent Technologies). Samples with a 28S/18S ribosomal peak ratio of 1.8 to 2.0 and a RIN number >7.0 were considered suitable for labeling. For samples meeting this standard, 2 μg of total RNA was used for cDNA synthesis using an oligo-dT-17 primer and the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen). Synthesis, linear amplification, and labeling of cRNA were accomplished by in vitro transcription using the MessageAmp aRNA Kit (Ambion) and biotinylated nucleotides (Enzo Diagnostics). Ten micrograms of labeled and fragmented cRNA were then hybridized to the Human HG-U133A2.0 GeneChip (Affymetrix) at 45°C for 16 hours. After washing, the chips were processed according to the manufacturer’s instructions and scanned with a high-numerical aperture and flying objective lens in the GS3000 scanner (Affymetrix). The image was quantified using GCOS version 1.4 (GeneChip Operating Software, Affymetrix).

Reverse transcriptase PCR

TaqMan Gene Expression Assays for MET, FGF-3, and 18s ribosomal RNA were purchased from Applied Biosystems. Gene expression was measured using the ABI Prism 7900HT Sequence Detection System from Applied Biosystems. Reverse transcriptase PCR (RT-PCR) of cDNA specimens was conducted as described previously (28).

siRNA

For siRNA experiments, cells were seeded in triplicate in 96-well plates at 5,000 cells per well in antibiotic-free complete medium and were allowed to adhere for 24 hours at 37°C. Thereafter, the cells were transfected with siGenome ON-TARGET plus FGF-3 or Non-Silencing Pool #3 siRNA (Dharmacon) according to the manufacturer’s instructions. Untransfected cells were used for control. After 48 hours, cell proliferation was determined using a BrdU cell proliferation assay.

Animal studies

Four- to 6-week-old nu/nu athymic BALB/c mice were obtained from the National Cancer Institute-Frederick Cancer Center and maintained in pressurized ventilated caging at the Sloan-Kettering Institute (New York, NY). All studies were done in compliance with Institutional Animal Care and Use Committee guidelines. Tumors were established by flank injection of 1 × 10^7 cells suspended with reconstituted basement membrane (Matrigel; Collaborative Research) at a ratio of 1:1 (volume). For efficacy studies, mice (5 per group) with established tumors were selected. Fourteen days after inoculation, mice were treated with SNX-5542, using the indicated doses. Tumor dimensions were measured with vernier calipers and tumor volumes were calculated using the formula: π/6 × larger diameter × (smaller diameter)^2.

Statistical analysis of xenograft experiments

Because of right-skewed distribution of tumor volume measurements, a logarithmic transformation of the tumor volume was performed. For data depiction, mean and corresponding 68% confidence intervals (CI) were
calculated and transformed back to the original scale (which would correspond to mean ± SE for normally distributed data). Group differences in tumor volume for the different cell lines and effects over time were modeled with a linear mixed model with repeated measures. A logistic transformation was applied to the dependent variable tumor volume to achieve homoscedasticity of residuals. Group differences, effect of time, and a group–time interaction were modeled and tested. All P values are 2-sided and P < 0.05 was considered significant. Calculations were performed using the statistical software package SAS(r) (SAS Institute Inc.; version 9.2).

Results

HSP-90 inhibition induces degradation of MET and other HSP-90 client proteins in tumor cells with MET amplification

We first assessed the effects of the synthetic HSP-90 inhibitor SNX-2112 on MET expression in 3 different MET-amplified tumor cell lines. As shown in Fig. 1A, SNX-2112 induced a dose-dependent degradation of MET in all cell lines, together with degradation of the HSP-90 client proteins HER-2, EGFR, and AKT. Degradation of MET was paralleled by complete dephosphorylation of MET, inhibition of downstream MAPK and AKT activation, as well as loss of cyclin D1 expression, indicating cell cycle arrest.

SNX-2112–induced dephosphorylation of MET, AKT, and MAPK occurs approximately 6 hours after initiation of treatment, whereas selective MET inhibition using the small molecule inhibitor PHA-665752 causes immediate dephosphorylation of MET and downstream signaling pathways

As shown in Fig. 1B, dephosphorylation of MET, AKT, and MAPK does not occur before 6 hours of SNX-2112 treatment, whereas PHA-665752, a highly selective small molecule inhibitor of MET, causes complete dephosphorylation of these proteins as early as 15 minutes after onset of treatment. However, in contrast to the HSP-90 inhibitor, PHA-665752 does not affect the total levels of MET, AKT, HER-2, and EGFR within the cells. After 24 hours of treatment, complete loss of cyclin D1 expression is observed both in SNX-2112- and PHA-665752-treated cells (Fig. 1B).

Effects of SNX-2112 on cell proliferation of tumor cells with MET amplification

Next, we assessed the effects of SNX-2112 on cell proliferation of MET-amplified cell lines. In all cells, a potent growth-inhibitory effect was observed. IC_{50} values for the individual cell lines were 25.2 (EBC-1), 30.3 (MKN-45), and 35.6 nmol/L (GTL-16; Fig. 1C).

SNX-2112 induces cell cycle arrest in tumor cells with MET amplification

Figure 2 shows the cell cycle data obtained after 24 hours of SNX-2112 treatment: Compatible with the cell proliferation results, SNX-2112 induced G1-arrest at a concentration of 50 nmol/L in all cell lines, whereas at higher concentrations (100 and 1,000 nmol/L) an increasing proportion of the cells accumulated in G2.

Efficacy of HSP-90 inhibition in a MET-amplified xenograft model

To study the antitumor efficacy of HSP-90 inhibition in MET-amplified tumors in vivo, nude mice bearing xenografts of GTL-16 and EBC-1 tumor cells were treated...
Figure 2. Cell cycle analysis. Cells were treated for 24 hours with increasing doses of SNX-2112, followed by flow cytometric analysis of the cell cycle distribution.
with increasing doses of SNX-5542, an orally bioavailable prodrug of SNX-2112. As shown in Fig. 3, treatment of mice with 50 mg/kg of SNX-5542 using a Monday–Wednesday–Friday schedule significantly inhibited growth of GTL-16 and EBC-1 xenografts as compared with the control group ($P < 0.0001$ and $P = 0.0003$, respectively) and was well tolerated without measurable toxicity. Importantly, increasing the dose of SNX-5542 to 75 mg/kg led to a further significant enhancement of the growth-inhibitory effect with partial regression of GTL-16 xenografts ($P < 0.0001$).

However, consistent with a previous report by Chandarlapaty and colleagues (25), SNX-5542 at the higher dose of 75 mg/kg also induced an average weight loss of 21.3% with 2 of 5 animal deaths by day 24. Consequently, the maximum SNX-5542 dose was reduced to 60 mg/kg for further experiments, albeit no significant enhancement of the growth-inhibitory effect as compared with the 50 mg/kg was noted ($P < 0.7115$; Fig. 3, bottom graph).

Creation of MET kinase inhibitor-resistant GTL-16 tumor cells

To assess whether HSP-90 inhibition maintains its antitumor efficacy in MET-amplified tumor cells with acquired resistance to a selective MET kinase inhibitor, we created GTL-16 cells resistant to the highly selective MET kinase inhibitor PHA-665752. Cells were cultured in vitro in increasing concentrations of PHA-665752 over a 6-month period, resulting in a resistant cell line (PR-GTL-16) that displayed uninhibited growth and cell cycle distribution even in the presence of 800 nmol/L PHA-665752 (Fig. 4A and B).
PR-GTL-16 cells display acquired expression of fibroblast growth factor-3 that is not detected in parental GTL-16 cells

Microarray analysis of PR-GTL-16 cells using the HG-U133A2.0 GeneChip revealed that 170 genes were differentially regulated with a fold-change cutoff of ±2 and a F-value cutoff of \( P < 0.005 \). Thirty-six of these genes were regulated at least 4-fold. The most highly regulated gene was FGF-3, which displayed a 64-fold increase of expression in PR-GTL-16 as compared with parental GTL-16 cells (Supplementary Table S1), making it the most likely candidate for the resistant phenotype of PR-GTL-16 cells although other changes (e.g., >4-fold upregulation of cyclin D2) should not be remain unmentioned. The microarray data were confirmed by RT-PCR analysis, in which FGF-3 mRNA was readily detectable in PR-GTL-16 cells, whereas it was below the detection limit in parental GTL-16 cells. Importantly, MET mRNA expression did not differ significantly between the cells, indicating that the resistance mechanism of PR-GTL-16 cells does not involve a change of MET expression (Fig. 4C).

FGF-3 confers resistance to MET inhibition through reactivation of AKT and MAPK signaling

Although parental GTL-16 cells displayed complete dephosphorylation of MET, AKT, and MAPK following PHA-665752 treatment, MET but not AKT and MAPK were dephosphorylated in PR-GTL-16 cells (Fig. 5A). On the basis of the microarray and RT-PCR results, we hypothesized that autocrine FGF-3 signaling could be responsible for sustained AKT and MAPK activation in PR-GTL-16 cells. This was supported by the observation that exogenous FGF-3 led to rephosphorylation of AKT and MAPK in parental GTL-16 cells and was able to rescue them from PHA-665752-mediated growth inhibition (Fig. 5A and B, lanes 5). Furthermore, siRNA-mediated knockdown of FGF-3 in combination with PHA-665752 (but neither treatment alone) significantly inhibited proliferation of PR-GTL-16 cells (Fig. 5C) and induced complete dephosphorylation of AKT and MAPK (data not shown). Finally, combined blockade of MET and FGF receptor (FGFR) signaling using PHA-665752 together with either of 2 selective FGFR inhibitors (PD173074 or PD330361) led to a dose-dependent inhibition of PR-GTL-16 proliferation, further illustrating FGF-3/FGFR-mediated signaling as the central mechanism of resistance to MET kinase inhibition in PR-GTL-16 cells (Fig. 5D). To assess in more detail which FGFRs mediate the FGF-3 effect in PR-GTL-16 cells, the expression of FGFR-1 and -2 (the receptors with the highest affinity for FGF-3; ref. 29) was determined using quantitative RT-PCR. Importantly, PR-GTL-16 cells were found to express both FGFR-1 and -2 mRNA, which strongly suggests that both receptors contribute to the FGF-3 rescue effect in these cells. This was further confirmed by FGFR-1/2 gene silencing using shRNA constructs (data not shown).

HSP-90 inhibition maintains its antitumor efficacy in MET-amplified tumor cells with acquired resistance to a MET kinase inhibitor

Having established a MET-amplified tumor cell line with acquired resistance to MET kinase inhibition, we next sought to determine whether inhibition of HSP-90 maintains its antitumor efficacy in the resistant cells. Figure 6A illustrates that treatment of PR-GTL-16 cells with SNX-2112 led to degradation of MET, AKT, EGFR, and HER-2 as in parental GTL-16 cells. SNX-2112 also induced dephosphorylation of MET, AKT, and MAPK together with loss of cyclin D1 expression, cell cycle arrest, and induction of apoptosis (Fig. 6B and D). The sensitivity of PR-GTL-16 cells toward SNX-2112 was comparable albeit slightly lower than that of parental GTL-16 cells [IC\(_{50}\) = 57.5 nmol/L [PR-GTL-16] versus 35.6 nmol/L (parental GTL-16); Fig. 6B and C]. We also tested the in vivo efficacy of SNX-5542 in nude mice bearing xenografts of PR-GTL-16 cells. As shown in Fig. 6E, SNX-5542 significantly inhibited growth of PR-GTL-16 xenografts, similar to its effect in parental GTL-16 xenografts.

Efficacy of HSP-90 inhibition in MET-amplified NCI-H820 cells

Recently, McDermott and colleagues (30) have shown that secondary activation of the EGFR signaling pathway is another mechanism of resistance toward MET kinase inhibition in MET-amplified tumor cells. To assess whether HSP-90 inhibition would also be effective in such MET-amplified tumor cells with secondary activation of EGFR signaling, SNX-2112 was tested in lung cancer-derived NCI-H820 cells that harbor both MET amplification and an activating T790M EGFR mutation. As shown in Supplementary Fig. S2, neither gefitinib (a selective EGFR inhibitor) nor PHA-665752 had an effect on cell proliferation in these cells whereas SNX-2112 led to a >50% inhibition of growth after 24 hours of treatment.

Discussion

The aim of this study was to assess the effects of HSP-90 inhibition using the novel synthetic HSP-90 inhibitor SNX-2112 in tumor cells with MET amplification. We have found that HSP-90 inhibition induces degradation of MET, inhibition of MET and downstream PI3K/AKT and Ras/Raf/MEK/MAPK signaling as well as cell cycle arrest in 3 different MET-amplified tumor cell lines (EBC-1, GTL-16, and MKN-45). We were also able to show that SNX-5542, an orally bioavailable prodrug of SNX-2112, has significant antitumor efficacy in vivo in nude mice bearing MET-amplified tumor xenografts. Notably, HSP-90 inhibition maintained its in vitro and in vivo efficacy against MET-amplified tumor cells resistant to the highly specific MET kinase inhibitor PHA-665752, suggesting that HSP-90 inhibition could be a particularly valuable strategy in MET-amplified tumors with acquired resistance to selective MET kinase inhibition.
Tumor cells harboring amplification of the MET oncogene are largely dependent on constitutive activation of the MET kinase for proliferation and survival, which renders them highly sensitive toward targeted MET inhibition (6, 7, 19). Several small molecule MET inhibitors have been developed and have recently entered early-stage clinical trials (31, 32). However, as with other highly selective kinase inhibitors (e.g., gefitinib and imatinib), development of acquired resistance is likely. Here, we show that de novo FGF-3 expression is a possible mechanism of resistance to MET inhibition.

Figure 5. FGF-3 stimulates AKT and MAPK phosphorylation and confers resistance to PHA-665752–mediated growth inhibition. A, GTL-16 and PR-GTL-16 cells were treated for 24 hours with 400 nmol/L PHA-665752 with or without addition of 0.1 μmol/L recombinant FGF-3, followed by Western blot analysis. B, cells were treated as in A, followed by a BrdU cell proliferation assay. Data are depicted as mean ± SE. C, PR-GTL-16 cells were transfected with FGF-3 siRNA and concomitantly treated with 400 nmol/L PHA-665752 for 48 hours. Thereafter, cell proliferation was measured using a BrdU cell proliferation assay. For control, cells were transfected with nonsilencing siRNA or left untransfected. Data are depicted as mean ± SE. D, PR-GTL-16 cells were treated with increasing doses of the selective FGF receptor (FGFR) inhibitor PD173074, alone or in combination with 400 nmol/L PHA-665752. In a second set of experiments, PD173074 was replaced by PD330361, another selective small molecule FGFR inhibitor. After 24 hours, cell proliferation was measured using a BrdU cell proliferation assay. Open squares, FGFR inhibitor alone; filled squares, FGFR inhibitor in combination with PHA-665752. Data are depicted as mean ± SE.
resistance to the selective MET kinase inhibitor PHA-665752 in MET-amplified GTL-16 tumor cells. In the resistant cell line (PR-GTL-16), FGF-3, which displays tumorigenic potential in murine breast and colorectal cancer models (33–37), maintains AKT and MAPK phosphorylation even when MET is completely inhibited, whereas knockdown of FGF-3 or selective inhibition of FGFR signaling using either of the 2 selective small molecule FGFR inhibitors (PD173074 or PD330361) largely restores PHA-665752-sensitivity.

Figure 6. HSP-90 inhibition maintains its in vitro and in vivo antitumor efficacy in PR-GTL-16 cells. A, PR-GTL-16 cells were treated with SNX-2112 at the indicated doses. After 24 hours, Western blot analysis was performed. B, cells were treated as in A, followed by cell cycle analysis. C, PR-GTL-16 and GTL-16 cells for comparison were treated with increasing doses of SNX-2112 for 24 hours, followed by a BrdU cell proliferation assay. D, GTL-16 and PR-GTL-16 cells were treated with increasing doses of SNX-2112 as indicated, followed by Annexin V/PI staining and flow cytometric analysis. Apoptotic cells: cells staining positive for Annexin V (including early apoptotic cells (Annexin V+/PI−) and late apoptotic cells (Annexin V+/PI+)). Necrotic cells: cells staining positive for PI without staining for Annexin V (Annexin V−/PI+). Viable cells: cells staining negative for both Annexin V and PI (Annexin V−/PI−). E, nude mice (5 per group) with established PR-GTL-16 xenografts were treated with the indicated doses of SNX-5542 on a Monday–Wednesday–Friday schedule. The tumor volume was measured as described in Materials and Methods. Data are depicted as mean ± 68% CI to account for right-skewed data distribution. This would correspond to a depiction as mean ± SE in the case of normal distribution. P values are from the regression model.
Our data add to the growing body of evidence that primary or secondary activation of parallel growth factor receptor signaling resulting in sustained PI3K/AKT and/or Ras/Raf/MEK/MAPK pathway activation is a mechanism of resistance to selective tyrosine kinase inhibition in oncogene-addicted cancer cells. Secondary amplification of MET confers resistance to the EGFR inhibitor gefitinib in EGFR-mutant non–small-cell lung cancer cells (38), loss of insulin-like growth factor (IGF) binding protein-3 expression with consecutive activation of the IGF receptor pathway mediates gefitinib resistance in A431 cells with EGFR amplification (39), and an autocrine FGF-2 signaling loop is associated with resistance to targeted EGFR inhibition in a subset of non–small-cell lung cancer cells (40). Furthermore, it has recently been showed by our group that HER-kinase activation confers resistance to targeted MET inhibition in MET-amplified gastric cancer cells (19).

Because oncogene-addicted tumor cells may readily overcome the effects of highly selective kinase inhibition through activation of redundant survival pathways, solitary inhibition of the oncogenically activated kinase is unlikely to exhibit long-term antitumor efficacy in these malignancies. Instead, the therapeutic strategy should take into account both oncogenic kinase activation as well as potential signaling pathway redundancy, that is, by simultaneously inhibiting multiple key signaling molecules. Conceivably, such an approach would decrease the likelihood of resistance as well as maintain antitumor efficacy in tumors that have become resistant to selective kinase inhibitor treatment.

A large number of oncogenic receptor tyrosine kinases and downstream signaling molecules require HSP-90 for conformational stability, including HER-2, EGFR, v-Src, Raf1, cyclin-dependent kinase-4, and AKT (10–17). Given the important roles played by these HSP-90 clients in signal transduction, proliferation, and survival, inhibition of HSP-90 has emerged as a potential antitumor treatment strategy (18). Its underlying mechanism involves proteasomal degradation of HSP-90 client proteins leading to disruption of the tumor cell signaling network—both at the receptor tyrosine kinase and downstream signaling level. HSP-90 inhibition therefore exemplifies the above-mentioned concept of multitargeted signaling pathway inhibition, making it a highly attractive strategy for the treatment of oncogene-addicted malignancies (41). Indeed, it could be showed that oncogene-addicted tumors (e.g., HER-2-amplified breast cancers or tumors with mutational activation of the EGFR) are particularly sensitive to HSP-90 inhibition both in vitro and in vivo (12, 15, 25).

In MET-amplified cells, a large number of HSP-90 clients (e.g., Raf-1, AKT and EGFR) are activated by MET either through MET-dependent downstream signaling or receptor cross talk (5–7, 19, 38). Moreover, recent studies have indicated that MET itself is an HSP-90 client protein (20–24). We therefore hypothesized that MET oncogene-addicted tumors may be equally amenable to the effects of HSP-90 inhibition. Indeed, our study shows that MET-amplified tumor cells are highly susceptible toward the antitumor effects of HSP-90 inhibition, with a sensitivity that is largely identical to that of HER-2–amplified breast cancer cells.

Of note, HSP-90 inhibition maintained its in vitro and in vivo antitumor efficacy in PR-GTL-16 cells with acquired resistance to the selective MET kinase inhibitor PHA-665752. In these cells, FGFR-3 (through its receptors FGFR-1 and -2) induces sustained activation of the PI3K/AKT and Ras/Raf/MEK/MAPK pathways even when MET is completely inhibited, suggesting that combinatorial signal transduction blockade is required for antitumor activity. Due to its multitargeted effect including up- (e.g., MET) and downstream (e.g., PI3K/AKT and Ras/Raf/MEK/MAPK) signaling molecules, which are also targeted by FGF-3, HSP-90 inhibition was able to simultaneously abrogate MET, PI3K/AKT, and Ras/Raf/MEK/MAPK signaling in the resistant cells, inducing growth inhibition both in vitro and in vivo at levels largely comparable to parental cells.

Recently, it could be shown that secondary activation of EGFR signaling is another mechanism of resistance to targeted MET inhibition in MET-amplified tumor cells (30). To assess whether HSP-90 inhibition using SNX-2112 would also be effective in such tumor cells that display both MET amplification as well as secondary activation of EGFR signaling, MET-amplified NCI-H820 cells that also harbor an activating T790M EGFR mutation were used as an in vitro model (42). As shown in Supplementary Fig. S2, these cells are resistant against both EGFR and MET inhibition, whereas a greater than 50% inhibition of growth is observed on treatment with SNX-2112. Importantly, our data suggest that HSP-90 inhibition could be a promising therapeutic strategy not only to overcome different mechanisms of MET kinase inhibitor resistance (e.g., secondary activation of FGFR or EGFR signaling) but also to treat EGFR-mutant non–small-cell lung cancers that have become resistant to EGFR inhibition through secondary amplification of MET (38).

In conclusion, our study provides a strong rationale for the use of HSP-90 inhibition in tumors that have become resistant to selective tyrosine kinase inhibition and further illustrates that combinatorial signal transduction blockade offers significant advantages over highly selective kinase inhibition in oncogene-addicted malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by a grant from the American Society of Colon and Rectal Surgeons. T. Bachleitner-Hoffmann was supported by postdoctoral research grants from the Max Kade Foundation, New York and the Austrian Surgical Society. M.R. Weiser was supported by a Career Development Award from the American Society for Clinical Oncology.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 29, 2010; revised September 28, 2010; accepted October 18, 2010; published online January 5, 2011.
References


Antitumor Activity of SNX-2112, a Synthetic Heat Shock Protein-90 Inhibitor, in MET-Amplified Tumor Cells with or without Resistance to Selective MET Inhibition

Thomas Bachleitner-Hofmann, Mark Y. Sun, Chin-Tung Chen, et al.

Clin Cancer Res 2011;17:122-133.

Updated version Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/17/1/122

Supplementary Material Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2011/01/03/17.1.122.DC1

Cited articles This article cites 41 articles, 24 of which you can access for free at: http://clincancerres.aacrjournals.org/content/17/1/122.full#ref-list-1

Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/17/1/122.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.