Anti-tumor activity of WK88-1, a novel geldanamycin derivative, in gefitinib-resistant non-small cell lung cancers with Met amplification

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Although epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have been introduced for the treatment of non-small cell lung cancer (NSCLC), the emergence of secondary T790M mutation in EGFR or amplification of the Met proto-oncogene restrain the clinical success of EGFR-TKIs. Since heat shock protein-90 (Hsp90) stabilizes various oncoproteins including EGFR and c-Met, the inhibition of Hsp90 activity appears as a rational strategy to develop anticancer drugs. Despite preclinical efficacy of geldanamycin-ansamycin (GA)-derivatives containing benzoquinone moiety as Hsp90 inhibitors, the hepatotoxicity of these GA-derivatives restricts their therapeutic benefit. We have prepared WK-88 series of GA-derivatives, which lack the benzoquinone moiety. In this study, we have examined the anticancer effects of WK88-1 in Met-amplified- and gefitinib-resistant (HCC827GR) NSCLC cells and its parental HCC827 cells. Treatment with WK88-1 reduced the cell viability in both HCC827 and HCC827GR cells, which was associated with marked decrease in the constitutive expression of Hsp90 client proteins, such as EGFR, ErbB2, ErbB3, Met and Akt. Moreover, WK88-1 attenuated phosphorylation of these Hsp90 client proteins and reduced the anchorage-independent growth of HCC827GR cells. Administration of WK88-1 did not cause hepatotoxicity in animals and significantly reduced the growth of HCC827GR cells xenograft tumors in nude mice. Our study provides evidence that ErbB3 might be a client for Hsp90 in Met-amplified NSCLCs. In conclusion, we demonstrate that inhibition of Hsp90 dampens the activation of EGFR- or c-Met-mediated survival of Met-amplified NSCLCs and that WK88-1 as a Hsp90 inhibitor alleviates gefitinib resistance in HCC827GR cells.

Lung cancer is one of the most prevalent malignancies and a leading cause of death worldwide. About 85% cases of all lung cancers are non-small cell lung cancer (NSCLC), which is characterized by multiple mutations in the gene encoding epidermal growth factor receptor (EGFR).1) Since the mutated EGFR functions as a constitutively active receptor tyrosine kinase (RTK), several small-molecule tyrosine kinase inhibitors (TKI) have been developed as chemotherapeutic agents for the treatment of NSCLC.2–5) TKIs, such as erlotinib (Tarceva) and gefitinib (Iressa) have been shown to specifically target EGFR and eradicate NSCLCs without causing nonspecific side-effects.6,7) Despite high selectivity of action and proven initial clinical success of TKIs, several studies have demonstrated that acquired resistance to TKIs develops in many patients within a couple of years.8,9) Acquired resistance has been shown to be mainly associated with two genetically conferred mechanisms – a secondary T790M mutation in EGFR and the Met amplification.10–12)

Several strategies have been proposed to overcome the acquired chemoresistance of NSCLC. Recently, irreversible EGFR-TKIs have been developed to inhibit the activity of T790M-EGFR,13,14) and combined treatment of NSCLC with EGFR-TKIs and Met-TKIs has been proposed to overcome resistance induced by c-Met amplification.15) Specifically, these approaches have been based on inhibiting the kinase activity of mutant EGFR or Met by selective inhibitors that target mutant receptor conformations, thereby modulating the EGFR or c-Met downstream signaling pathways.14,16) However, these therapeutic approaches often result in renewed drug resistance, because oncogene-addicted tumor cells can readily thwart the anticancer effects of a mono-targeted therapy by activating alternative survival pathways.17,18) Thus a rational strategy to overcome acquired chemotherapy resistance is to develop drugs that can target multiple cancer-related biochemical pathways.

Heat shock protein-90 (Hsp90), a component of a multi-chaperone complex, plays a key role in the stabilization and
maturation of various wild type and/or mutant forms of proteins, such as EGFR, c-Met, ErbB2, Raf1, and Akt.\(^{19–22}\) The stabilization and overexpression of these oncoproteins have been implicated in the development and progression of cellular transformation.\(^{23–25}\) The disruption of Hsp90 chaperone activity induces degradation of these client proteins via the ubiquitin-proteasome pathway. Thus, the blockade of Hsp90 activity may inhibit diverse oncogenic signaling pathways. Accumulating evidence indicates that pharmacological inhibition of the Hsp90 activity has emerged as a promising strategy for cancer therapy.\(^{26}\) It is interesting to note that Hsp90 is constitutively expressed in tumor cells at 2–10 fold higher levels than in the normal cells.\(^{27}\) Moreover, Hsp90 exists in tumor cells as an ATP-bound active multi-chaperone complex, while it remains in normal cells in a latent or uncomplexed state.\(^{28}\) This phenomenon coincides with the finding that tumor cell-derived Hsp90 complexes have higher binding affinity to Hsp90 inhibitor, 17-AGG, a 17-allyl amino derivative of geldanamycin ansamycin (GA), as compared with Hsp90 present in normal cells.\(^{28}\) This higher affinity of tumor-derived Hsp90 for its inhibitor compounds suggests the potential selectivity of Hsp90 inhibition for cancer cells over normal cells. Although Hsp90 is an essential chaperone protein for all cells, tumor cells exhibit greater dependence on Hsp90’s chaperoning function to restructure numerous unfolded and mutated proteins including EGFR and c-Met, which contribute to the development of chemoresistance in NSCLC. Thus, the targeted inhibition of Hsp90 activity would be a rational approach to overcome acquired resistance and to achieve the therapeutic goal.

Practically, Hsp90 inhibitors including GA and its derivatives have been reported to show strong antitumor effect on the growth of NSCLCs with T790M mutation of EGFR\(^{20,29,30}\) Despite effective preclinical anticancer activities of GA and its derivatives, the clinical success of these molecules are thwarted by their hepatotoxic properties and poor in vivo stability.\(^{31,32}\) Although other classes of Hsp90 inhibitors such as purine scaffold inhibitors or diarylpyrazole compounds have been investigated,\(^{33,34}\) more improved GA-modified Hsp90 inhibitors lacking potential side-effects are being sought. Previous reports suggest that the undesirable toxicity of GA and its derivatives results from the “off target” effects of the benzoquinone moiety.\(^{35,36}\) Therefore, the GA derivative that lacks a benzoquinone moiety may be devoid of toxic effects. In accord with this, we recently reported the development of non-benzoquinone GA (e.g., WK88-1, WK88-2 and WK88-3) derivatives by a mutasynthetic approach and directed biosynthetic method using genetically engineered Streptomyces hygroscopicus (Fig. 1).\(^{37,38}\) In the present study, we report the molecular mechanisms of antitumor effects of WK88-1, a new Hsp90 inhibitor, in gefitinib-resistant NSCLC cells with amplified c-Met.

**Materials and Methods**

**Materials.** Antibodies specific for phospho-EGFR (Tyr1068; #3777), Met (#4560), phospho-Met (Tyr1234/1235; #3077), ErbB3 (#4754), phospho-ErbB3 (#4791), Akt (#4691), phospho-Akt (Ser473; #4060), Hsp90 (#4874), Hsp70 (#4872), Erk1/2 (#4695), phospho-Erk1/2 (Thr202/Tyr204; #4370), cleaved Caspase-3 (#9661), PARP (#9542) and β-actin (#4970) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibody specific for EGFR was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA; sc-03). Gefitinib was purchased from LC Laboratories (Woburn, MA, USA; #G-4408). Geldanamycin was obtained from Enzo Life Sciences.

Fig. 1. Schematic representation of production of the benzoquinone and non-benzoquinone GA analogs. DHQ3 (15-hydroxy-17-demethoxyreblastatin) was purified in a combinational mutation with site-directed mutagenesis of the first dehydratase domain of the geldanamycin polyketide synthase (PKS) gene (gelA) and a post-PKS modification gene (gel7) of Streptomyces hygroscopicus JCM4427. WK88-1 (18-dehydroxyl-17-demethoxyreblastatin), WK88-2 (18-hydroxyl-17-demethoxyreblastatin), and WK88-3 (18-hydroxyl-17-demethoxy-4,5-dehydroreblastatin) were purified from a culture of S. hygroscopicus AC2, in which the AHBA synthase gene was disrupted by the kanamycin-resistance gene, supplemented with 3-aminobenzoic acid.
Fetal bovine serum (FBS), streptomycin, and penicillin were obtained from Thermo Scientific (South Logan, UT, USA). Non-benzoquinone geldanamycin analogs were produced by following a mul-
tasynthetic approach and a directed biosynthetic method. DHQ3, a 15-hydroxyl-17-demethoxy non-benoquino-
analog, was prepared from a genetically engineered strain (AC15) of Streptomyces hygroscopicus. HCC827 (del E746_A750 in exon 19)(39) were maintained in RPMI-1640 with L-glutamine supple-
mented with 10% FBS, penicillin and 20 \( \mu \)g/mL streptomycin, and maintained at 37°C in a 5% CO\(_2\) incubator for 6 to 7 days, and the colonies were counted under light microscope.

**Flow cytometry.** Cell apoptosis was detected with an FITC Annexin V Apoptosis detection kit I (BD Biosciences Pharmingen) in accordance with the supplied protocols. After exposure to indicated compounds for 24 h, cells were collected. Cells were washed twice with cold PBS and then resuspended in 1× binding buffer at a concentration of 1 × 10\(^6\) cells/mL. 5 \( \mu \)L of FITC Annexin V and PI were added to 100 \( \mu \)L of suspended cells (1 × 10\(^6\) cells), and the cells were incubated for 15 min at RT in the dark. The analysis was done with a BD FACSuite flow cytometer with BD FACSuite Software. The fraction of cell population in different quadrants was analyzed using quadrant statistics.

**Animals.** For in vivo xenograft assay, male BALB/c-nu athymic nude mice (5 weeks old) were obtained from Orient (Seoul, South Korea) and maintained under specific pathogen-free conditions based on the guidelines established by the Seoul National University. Animals were acclimated for 1 week before the study and housed in climate-controlled units with a 12-h-light/12-h-dark cycle. For in vivo hepatotoxicity assay, male C57BL/6 mice (7 weeks old) were obtained from Koatech (Pyungtaek, Gyeonggi, South Korea), housed under specific pathogen-free conditions and acclimated to the local environment for 1 week before use. The in vivo experiments were conducted using protocols approved by the Institutional Animal Care and Use Committee at Korea Research Institute of Bioscience and Biotechnology (KRBIB).

**Xenograft mouse model.** Mice were divided into two groups for each cell line: (i) vehicle group (n = 10); (ii) 1 mg/kg of WK88-1 (n = 10). HCC827GR (1 × 10\(^6\) cells/100 \( \mu \)L) cells were suspended in RPMI-1640 medium and inoculated with 100 \( \mu \)L matrigel subcutaneously into the right flank of each mouse. Vehicle or WK88-1 was injected intraperitoneally three times per week. Tumor volume was calculated according to a standard formula: tumor volume (mm\(^3\)) = (length × width \(^2\) × height × 0.52). Tumor volume was measured every 3 or 4 days, and tumor weight was recorded after excision on the day of termination of the experiment. Mice were monitored until tumors reached 1 cm\(^3\) total volume, at which time mice were euthanized and tumors extracted.

**Hepatotoxicity assay.** C57BL/6 mice were treated intravenously with vehicle, GA (10 mg/kg), WK88-1 (10 or 30 mg /kg), WK88-2 (10 or 30 mg/kg) or WK88-3 (10 or 30 mg/kg) twice at 24 h intervals (n = 5). Body weight was recorded daily. At the end of experiment, blood was collected from posterior vena cava and plasma was prepared for biochemical analysis. The level of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) in plasma was measured using biochemical analyzer (AU400; Olympus, Tokyo, Japan). For in vivo experiments, GA, WK88-1, WK88-2 and WK88-3 was firstly dissolved in two volumes of absolute ethanol. After the compounds were completely dissolved, two volumes of Cremophor EL were added and final dosing solution was prepared by adding six volumes of 20% hydroxypropyl \( \beta \)-cyclodextrin.

**Statistical analysis.** Quantitative data are presented as mean value ± SD unless indicated otherwise. The statistical significant of compared measurements was measured using the
Student’s $t$-test and $P < 0.05$ or less calculated from two-tailed test was considered significant.

**Results**

**WK88-1, a non-benzoquinone GA derivative, suppresses the proliferation of gefitinib-resistant NSCLC cells with Met amplification.** We have previously designed and synthesized non-benzoquinone GA derivatives by following mutasynthetic and directed biosynthetic approaches.\(^{(37,38)}\) As shown in Figure 1, DHQ3, a 15-hydroxy-17-demethoxyreblastin, was prepared from a genetically engineered strain (AC15) of *S. hygroscopicus*\(^{(38)}\) and WK88-1, WK88-2, and WK88-3 were purified from a culture of *S. hygroscopicus* AC2, in which the AHBA synthase gene was disrupted by the kanamycin-resistance gene, supplemented with 3-aminobenzoic acid.(Fig. 1).\(^{(37)}\) In this study, we examined the possible effects of DHQ3 and the WK88 series of non-benzoquinone GA derivatives in alleviating gefitinib resistance in NSCLC. For this purpose, we used a gefitinib-sensitive HCC827 cells and gefitinib-resistant HCC827GR cell line harboring Met gene amplification. As reported, our data also showed that HCC827 cells were highly sensitive to exposure to gefitinib, whereas HCC827GR cells were relatively resistant to gefitinib treatment (Fig. 2a,b). Given that Hsp90 inhibition seems promising to overcome gefitinib resistance, we first assessed the anti-proliferative effects of these compounds in these NSCLC cell lines. Our data revealed that a potential growth-inhibitory effect was observed in NSCLCs, which was treated with WK88 compounds or GA, whereas DHQ3 didn’t show any desirable effects (Fig. 2a,b). Notably, this anti-proliferative effect of WK88 compounds was evidently observed in HCC827GR cells as well as HCC827 cells, suggesting that WK88 compounds might be a potential alternative of GA to overcome acquired resistance to gefitinib in NSCLCs.

Since hepatotoxicity is a major obstacle in developing GA derivatives as potential chemotherapeutic agents, finding the nontoxic Hsp90 inhibitor might be an important task. Therefore, hematological biochemistry tests were performed to examine hepatotoxicity of WK88 compounds in mice. Our data revealed that intravenous injection with WK88 compounds did not adversely affect body weight in C57BL/6 mice (Table 1). Moreover, biochemical evaluation measured by glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels clearly showed that WK88-1 and WK88-2 caused no detectable hepatotoxicity, whereas strongly increased GOT/GPT levels were detected in mice treated with WK88-3 (30 mg/kg body weight) (Table 1). These increased levels of GOT/GPT in WK88-3-injected mice are comparable to those in GA-injected mice considering lower

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**Fig. 2.** WK88-1 suppresses the proliferation of gefitinib-resistant non-small cell lung cancers (NSCLCs) via the downregulation of oncogenic RTKs. Gefitinib-sensitive HCC827 cells (a) and resistant HCC827GR cells (b) were treated with the indicated concentration of GA derivatives for 3 days, and cell proliferation was estimated using the MTS assay. Cell viability relative to controls was determined after 3 days. Data shown are the representative of 5 independent experiments. Error bars represent the mean ± SD. Statistical significance was determined by the Student’s $t$-test ($***P < 0.001$). Effect of GA derivatives on the expression or activity of EGFR, Met, ErbB3, and downstream proteins (c). Cells were treated with 1 μM GA derivatives for 24 h and whole cell lysates were assayed by Western blot. β-Actin was used as a loading control. GA, Geldanamycin; G, Gefitinib; W1, WK88-1; W2, WK88-2, W3, WK88-3.
amplification leads to persistent activation of PI3K maintaining ErbB3 phosphorylation in an EGFR- and ErbB2-
expected, treatment with 1
examined their effects by analyzing the expression of Hsp90’s
which WK88 compounds inhibit cell growth in NSCLCs, we
in gefitinib-sensitive HCC827 cells, but not in gefitinib-resis-
tant HCC827GR cells (Fig. 2c). Among tested compounds, WK88-
⁄
\( ^1 \) have revealed that Met
astatic activity, we next performed migration and invasion
assay in HCC827 and HCC827GR cells. As shown in
Figure 4(a), treatment with gefitinib markedly reduced the migra-
tion and invasion in HCC827 cells, but not in gefitinib-
resistant HCC827GR cells (Fig. 4a,b). However, treatment

Table 1. Changes in body weight and plasma glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) level after treatment with geldanamycin-ansamycin (GA) and non-benzoquinone GA derivatives

| Group (n = 5) | Doses (mg/kg) | Weight change after treatment | GOT (IU/L) | GPT (IU/L) |
|--------------|--------------|-------------------------------|------------|------------|
|              |              | Day 0            | Day 1 | Day 2 |           |            |
| Vehicle Control | 0           | 100.0 ± 0.0 | 99.3 ± 2.7 | 99.2 ± 3.3 | 68.0 ± 15.7 | 33.0 ± 5.7 |
| Geldanamycin (GA) | 10          | 100.0 ± 0.0 | 99.8 ± 1.8 | 93.7 ± 6.7 | 23160.0 ± 6523.9 | 18572.0 ± 5735.4 |
| WK88-1 (W1) | 10           | 100.0 ± 0.0 | 99.8 ± 2.4 | 100.6 ± 3.7 | 53.2 ± 12.0 | 31.0 ± 5.5 |
|              | 30           | 100.0 ± 0.0 | 101.4 ± 1.7 | 98.6 ± 7.9 | 66.0 ± 24.3 | 36.0 ± 6.5 |
| WK88-2 (W2) | 10           | 100.0 ± 0.0 | 99.8 ± 0.7 | 102.7 ± 4.4 | 56.0 ± 4.2 | 34.0 ± 6.5 |
|              | 30           | 100.0 ± 0.0 | 101.8 ± 2.9 | 100.6 ± 2.1 | 70.0 ± 18.0 | 68.0 ± 54.4 |
| WK88-3 (W3) | 10           | 100.0 ± 0.0 | 100.5 ± 2.0 | 99.4 ± 6.6 | 72.5 ± 13.2 | 56.3 ± 18.9 |
|              | 30           | 100.0 ± 0.0 | 99.5 ± 2.5 | 96.4 ± 3.2 | 2869.0 ± 3521.7 | 3722.0 ± 4419.1 |

CS78L/C6 mice (n = 5) were treated intravenously with vehicle or the indicated concentrations of GA or GA derivatives (WK88-1, WK88-2, and WK88-3). Body weight was recorded daily and plasma level of GOT and GPT was measured by biochemical analysis. Values were expressed as mean ± SD of five determinations.

Fig. 3. WK88-1 augments the instability of oncogenic RTKs in non-small cell lung cancers (NSCLCs). Cells were treated with cycloheximide (CHX) in combination with WK88-1 for the indicated time and then subjected to Western blot (a). Proteasome inhibitor MG132 restored oncogenic RTKs after WK88-1 treatment. Cells were treated with MG132 for 2 h prior to WK88-1 treatment and then subjected to Western blot (b).
with WK88-1 strongly abrogated the migratory and invasive capacities of both gefitinib-sensitive and resistant cell lines (Fig. 4a,b). Notably, the representative microphotograph clearly shows that a significant inhibition of migration and invasion was observed in Met-amplified HCC827GR cells following treatment with WK88-1. The soft-agar colony formation assay also revealed that treatment with WK88-1 inhibited the anchorage-independent growth of HCC827GR cells, indicating that the malignant potential of HCC827GR cells might be blunted by WK88-1 (Fig. 4c).

WK88-1 induces apoptosis and inhibits xenograft tumor growth of gefitinib-resistant NSCLC cells in nude mice. Incubation with WK88-1, but not gefitinib, induced apoptosis in gefitinib-resistant HCC827GR cells in a concentration-dependent manner as revealed by Annexin-V staining and flow-cytometry analyses (Fig. 5a). Treatment with WK88-1 also increased the expression of cleaved caspase-3 and cleaved PARP (Fig. 5b), indicating the potential of WK88-1 in inducing death signaling in gefitinib-resistant NSCLCs with Met amplification. We next examined whether WK88-1 overcomes resistance to gefitinib in vivo. To investigate the in vivo antitumor activity of WK88-1, gefitinib-resistant HCC827GR cells were subcutaneously transplanted into nude mice. Treatment with WK88-1 (1 mg/kg body weight) caused a significant decrease in tumor volume (Fig. 5c) and weight (Fig. 5d), suggesting that treatment with WK88-1 could circumvent gefitinib resistance in NSCLCs.

Discussion

Intracellular signaling networks comprising multiple RTKs and their downstream molecules may confer the molecular complexity and compensatory pathway against TKIs-mediated inhibition of tumor cell growth. In particular, members of the EGFR family, including EGFR, ErbB2, ErbB3, ErbB4 and Met, have been reported to crosstalk with each other, thereby developing resistance to EGFR-TKIs. For example, NSCLCs with activating mutations in EGFR develop acquired resistance to EGFR-TKIs by recruiting Met and subsequent activation of ErbB3-PI3K-Akt axis, indicating a central role for ErbB3 in acquiring chemoresistance by tumor cells. Also, a recent report showed that inhibiting EGFR with gefitinib also inhibits phosphorylation of ErbB2, ErbB3, and Met in gefitinib-sensitive cells. Moreover, EGFR has been shown to regulate Met expression at multiple levels Consequently, it is crucial to define the exact mechanism by which drug resistance develops and find an integrative strategy for simultaneous blocking of both oncogene activation and potential redundant cell signaling pathways. Because of these signaling complexities and an increasing trend of drug-resistance, a

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molecular target that plays a central role in diverse oncogenic signaling pathways have long been sought. One of the promising anticancer drug targets is Hsp90, a molecular chaperone that promotes the proliferation and growth of cancer cells, at least in part, through the stabilization and maturation of various oncoproteins. In NSCLCs, Hsp90 has been shown to stabilize and activate various oncoproteins, including EGFR and MET, which are overexpressed in many NSCLCs. This activation contributes to the growth and survival of cancer cells. To overcome acquired resistance to EGFR inhibitors such as gefitinib, which is commonly used in NSCLCs, researchers have explored alternative targets such as Hsp90. One such approach is the use of small molecules that target Hsp90, such as KG16 or WK88-1, which have been shown to induce cell death in HCC827GR cells treated with gefitinib. These results suggest that targeting Hsp90 may be a promising strategy for overcoming acquired resistance to EGFR inhibitors in NSCLCs.
lize key oncogenic proteins including EGFR, ErbB2, Raf-1, Met, and Akt. Thus, attempts have been made to develop anticancer therapies, targeting Hsp90 because blockade of the Hsp90 activity would cause simultaneous inhibition of cell survival pathways mediated by aforementioned oncoproteins.

Although a number of GA-derivatives, such as 17-AAG and 17-DMAG have been developed as Hsp90 inhibitors with remarkable preclinical success, the major limitations of these molecules are their hepatotoxic activity, which is likely to be caused by the benzoquinone moiety present in their structure. We, therefore, have prepared several GA-derivatives lacking the benzoquinone group in their structure and evaluated their anticancer activities in both gefitinib-sensitive and-resistant NSCLCs. Among the non-benzoquinone GA-derivatives, WK88-1, was found to have no hepatotoxic effects, while the WK88-3 appears to show hepatotoxicity. Though WK88-2 did not exhibit hepatotoxicity but showed only a marginal effect in suppressing tumor NSCLC cells growth. We, therefore, further examined the role of details underlying the antitumor effects of non-benzoquinone GA-derivative, WK88-1.

Our data clearly shows that WK88-1 induces simultaneous degradation of RTKs and inhibition of downstream signaling pathways, thereby suppressing the proliferation of HCC827GR cells. This is further supported by the inhibitory effect of the compound on the growth of the gefitinib-resistant NSCLC cells, and the reduced growth of HCC827GR cells xenograft tumors in nude mice. Furthermore, the inhibition of the migration and invasion of HCC827GR cells by treatment with WK88-1 suggests that this novel Hsp90 inhibitor is effective in alleviating the EGFR-TKI resistance. Whereas WK88-1 showed antiproliferative effects in vitro, it would be worthwhile to examine whether the membrane permeability of WK88-1 and DHQ3 differs in relation to their chemical properties.

One of the molecular targets for overcoming EGFR-TKI resistance is to inactivate signaling mediated via Met, which has been implicated in a wide range of human malignancies inducing NSCLC. In addition, acquired resistance to EGFR-TKIs in NSCLC has been reported to emerge by recruiting Met and subsequent activation of ErbB3. Based on the crucial roles of ErbB3-Pi3K-Akt axis in Met-amplified HCC827GR cells, we sought to determine whether treatment with WK88-1 could diminish the expression levels of these oncoproteins. Our findings show that treatment with WK88-1 downregulates the protein levels of ErbB3 together with other well-known Hsp90 client proteins including EGFR, ErbB2, Met, and Akt, suggesting that ErbB3 is a potential substrate for Hsp90 as reported. Therefore, these findings provide insight as to how the treatment with WK88-1 may block alternative cell survival pathways in Met-amplified HCC827GR cell lines (Fig. 6).

In conclusion, our findings clearly demonstrate that treatment with WK88-1 lead to a significant reduction in vivo tumor growth in a mouse xenograft model as well as migration, invasion, and colony formation in gefitinib-resistant NSCLCs. Our data also reveal that intraperitoneal administration of WK88-1 in mice does not elicit any undesirable toxicity, suggesting that WK88-1 might be a less toxic inhibitor for Hsp90. Therefore, our data suggest that WK88-1 is a potential alternative of GA for overcoming acquired resistance to gefitinib in NSCLCs.

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Disclosure Statement

The authors have no conflict of interest.

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