Original Research

VER-155008, a small molecule inhibitor of HSP70 with potent anti-cancer activity on lung cancer cell lines

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Abstract

Lung cancer is the most common malignancy and exhibits significant morbidity and mortality worldwide. Among all lung cancer subtypes, non-small-cell lung cancer (NSCLC) accounts for the majority of all lung cancer cases. Although there have been intensive investigations on the underlying mechanism of NSCLC development and progression, the exact molecular basis is not well understood. Further insights on important molecular regulators of lung cancer are needed for development of novel therapeutics. The heat shock protein (HSP) family is a group of molecular chaperones that assist in protein folding, modification, and transportation. Different HSPs are essential for tumor cell survival by binding diverse client proteins and regulating homeostasis. In the current study, we sought to characterize HSP70 and HSP90 as potent regulators of NSCLC growth. Our results indicate that differential expression of HSP70 is associated with the malignant phenotype of NSCLC cell lines and plays an important regulatory role in NSCLC cell proliferation. Moreover, a specific inhibitor of HSP70, VER-155008 significantly inhibits NSCLC proliferation and cell cycle progression. We showed that this effect is largely abolished by HSP70 overexpression, indicating that the inhibitory effect of VER-155008 on cell growth is specifically through HSP70 inhibition. In addition, 17-AAD, an inhibitor of HSP90, exerts a potent synergistic effect on NSCLC proliferation with VER-155008. We also observed that inhibition of HSP70 by VER-155008 can sensitize A549 cells to ionizing radiation. These data provide proof-of-principle that VER-155008 can be a good candidate for NSCLC treatment and HSP machinery is a good target for developing NSCLC therapeutics.

Keywords: Heat shock protein 70, heat shock protein 90, VER-155008, 17-AAD, non-small-cell lung cancer

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Introduction

Lung cancer is the most commonly diagnosed cancer (1.61 million, 12.7% of the total) and leading cause of cancer-related deaths (1.38 million, 18.2% of the total) worldwide.[1] About 80% of total lung cancer cases are classified as non-small-cell lung carcinoma (NSCLC). The prognosis of NSCLC is very poor, having an overall five-year survival rate as low as 15% and without a notable decrease in mortality during the last three decades.[2,3] This is primarily due to the limited therapeutic effects of adjuvant postoperative chemotherapy in NSCLC patients. Less than half of NSCLC patients respond to chemotherapy initially, and there is frequent development of drug resistance in patients who do respond. Recent studies emphasize the importance of targeting multiple pathways to increase tumor cell sensitivity and to postpone the emergence of drug resistance. Therefore, novel drug targets involved in new pathways essential for NSCLC development are avidly needed for NSCLC patients.

The heat shock protein (HSP) family is a group of molecular chaperones that assist in protein folding, modification, and transportation. In conditions of cell stress, including elevated temperatures, nutrient deprivation, heavy metal exposure, oxidative stress, and exposure to cytotoxic agents, HSPs aid in cell survival. HSP70 is a basic member of HSPs and is important in cancer research and drug discovery. HSP70 overexpression is correlated with a wide range of tumors[4–6] and seems to be necessary for the survival of tumor cells. Additionally, expression levels of HSP70 in lung cancers are significantly higher than in peritumoral normal tissues.[7] Serum levels of HSP70 are also significantly elevated in patients with NSCLC compared with healthy individuals.[8]. Furthermore, recent studies demonstrate that selective depletion of HSP70 induces cell death in lung cancer cells[9] but not in normal lung cells.[10]

More interestingly, a few researches have demonstrated that HSP70 proteins can be induced by ionizing radiation (IR) in cells,[11,12] and elevated protein levels of HSP70 are

involved in radioresistance of cells.[13–16] The protective effects of HSP70 proteins might be due to their abilities that help DNA-damage repair.[15,17] All these information suggests that inhibition of HSP70 could improve radiosensitivity of cancer cells and may finally achieve a better clinical outcome.

So far, only a few compounds that specifically target HSP70 have been identified.[18]. However, their inhibitory activity on tumor growth seems attractive to cancer biologists.[19] Here in this study, we used a newly invented HSP70 inhibitor, VER-155008 which targets the ATPase binding domain of HSP70.[20] Previous reports indicate this compound induces apoptosis in myeloma cells[21] and HCT116 colon carcinoma cells[22] while having minimal effects on normal cells. So far, there is no study showing the effect of VER-155008 on growth of NSCLC cell lines. Therefore, in the present study, we tested the ability of VER-155008 to inhibit growth of NSCLC cell lines and explored the underlying molecular mechanisms of this effect. In addition, we tested the anti-cancer effects of VER-155008 in synergy with 17-AAD, an inhibitor of HSP90. Finally, we also tested the effect of VER-155008 in sensitizing NSCLC cells to IR.

Materials and methods

Reagents

Anti-HSP70 antibody (AB2787) and anti-GAPDH (AB8245) antibody were purchased from Abcam. Anti-Cyclin A antibody (SC751) and anti-CDK2 (SC6248) antibody were purchased from Santa Cruz Biotechnology, Inc. Antibodies to total Akt (4685), total ERK (4696), phosphorylated Akt (4058), and phosphorylated ERK (4376) were obtained from Cell Signaling. ViraPower Lentiviral Expression Kit was purchased from Invitrogen. 5-Bromo-2′-deoxyuridine (BrdU) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) were purchased from Sigma-Aldrich, Shanghai, China.

Cell culture

Human NSCLC cell lines, A549, and H1975 were obtained from American Type Culture Collection (ATCC) and cultured in F-12 K and RPMI-1640 medium, respectively. Human lung fibroblasts, WI38 cells, were also obtained from ATCC and cultured in DMEM medium. Medium was supplemented with 10% final concentration of fetal bovine serum, 1 U/mL of penicillin, and 1 μg/mL of streptomycin. For drug treatment, 25 μM of VER-155008 was applied on cells for two days if without specific indication.

Plasmids and transduction

Short hairpin RNA (shRNA) targeting HSP70 (NM_005345) was synthesized and cloned into pLKO.1 vector. The sense strand sequence for lentivirus-encoded HSP70 shRNA was [22948392]: 5′-GGACGAGTTTGAGCACAAG-3′. Lentiviral particles were produced from 293 FT cells using ViraPower Lentiviral Expression Kit according to manufacturer's protocol. Transduced cells were selected by treatment with puromycin (0.5 μg/mL) for at least three days before they were used in following experiments.

The coding DNA sequence of HSP70 (HSPA1A) was amplified from another plasmid (Addgene 15215) by PCR, and the fragment was inserted into pBABE-Puro vector. The empty vector or the plasmid encoding HSP70 was transfected into Phoenix Retroviral Expression System. Virus was applied onto target cells according to the standard protocol. The cells were subjected to drug selection for two days (1 μg/mL of puromycin) before they were used in the experiments.

Western blot

Thirty micrograms of protein for each sample were resolved on 8% or 12.5% SDS-PAGE gels. After transfer, membranes were incubated with primary antibodies at 4°C overnight. The dilution of antibodies used for all immunoblot assays in this study is 1:1000 except for GAPDH antibody (1:10000). Membranes were then washed with TBS-T three times and incubated with appropriate HRP-conjugated secondary antibodies at room temperature for 1 h. Membranes were subsequently washed with TBS-T four times and incubated with ECL Western Blotting Substrate (Promega, W1001). Films were exposed to the membranes in a dark room and then developed and fixed.

Cell viability assay

Cells were seeded at a density of 10,000 cells per well. After different drug treatments, 10 μL of MTT stock solution was added into each well and cells were incubated for 4 h at 37°C. SDS-HCl solution (100 μL) was added to each well and mixed thoroughly. Plates were then incubated at 37°C in the dark for 2 h with gentle shaking. Absorbance was read at 570 nm on a Microplate Reader.

BrdU staining

The cells are seeded on coverslips overnight. BrdU was added into culture medium to 10 μM and the cells were incubated for 1 h at 37°C. Then the cells were washed with PBS and fixed in 4% paraformaldehyde for 10 min. The cell were permeabilized with 0.2% Triton X-100 for 5 min and washed with PBS. The cells were treated with DnaseI (Invitrogen) for 30 min and then washed with PBS. The slides were incubated in anti-BrdU FITC for 30 min in the dark and then washed with PBS. The cells were stained with DAPI solution (1 μg/mL) for 5 min and washed with PBS and mounted on coverslips.

FACS

Non-adherent cells were collected into a conical tube. Adherent cells were trypsinized, combined with non-adherent cells, and centrifuged at 1000 rpm for 10 min. Cells were washed in cold PBS, centrifuged at 1000 rpm for 10 min at 4°C, fixed and permeabilized with ice-cold 70% ethanol, and incubated on ice for 6 h. Cells were then centrifuged for 10 min at 800 *g*, washed with PBS once, and resuspended in 0.5 mL of PI/Triton X-100 staining solution. Cells were incubated in the dark at 37°C for 30 min before

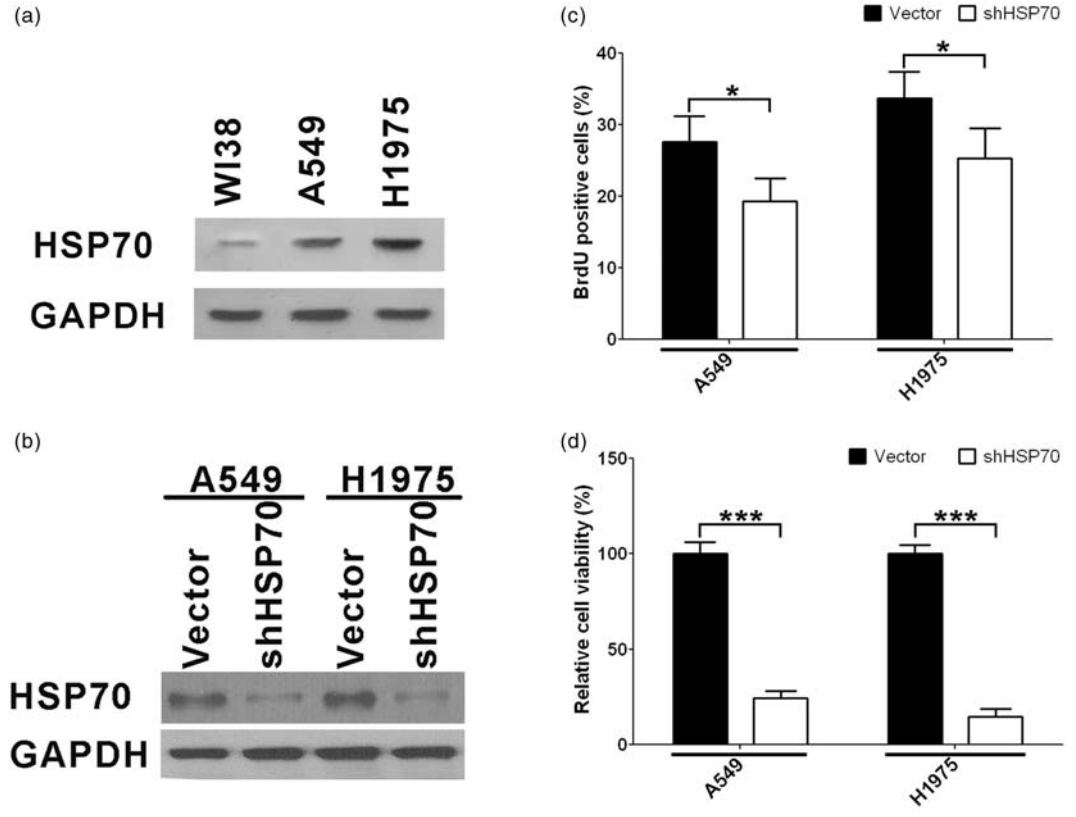

Figure 1 HSP70 knockdown inhibits proliferation of A549 and H1975 cells. (a) The expression levels of HSP70 in WI38, A549, and H1975 cells. GAPDH was used as loading control. (b) The expression levels of HSP70 in A549 and H1975 cells with and without HSP70 knockdown. (c) The percentages of BrdU-positive cells in A549 and H1975 cells with and without HSP70 knockdown. Results were presented as Mean ± SD, $n = 3$. (d) Relative cell viabilities of A549 and H1975 cells with and without HSP70 knockdown measured by MTT assay

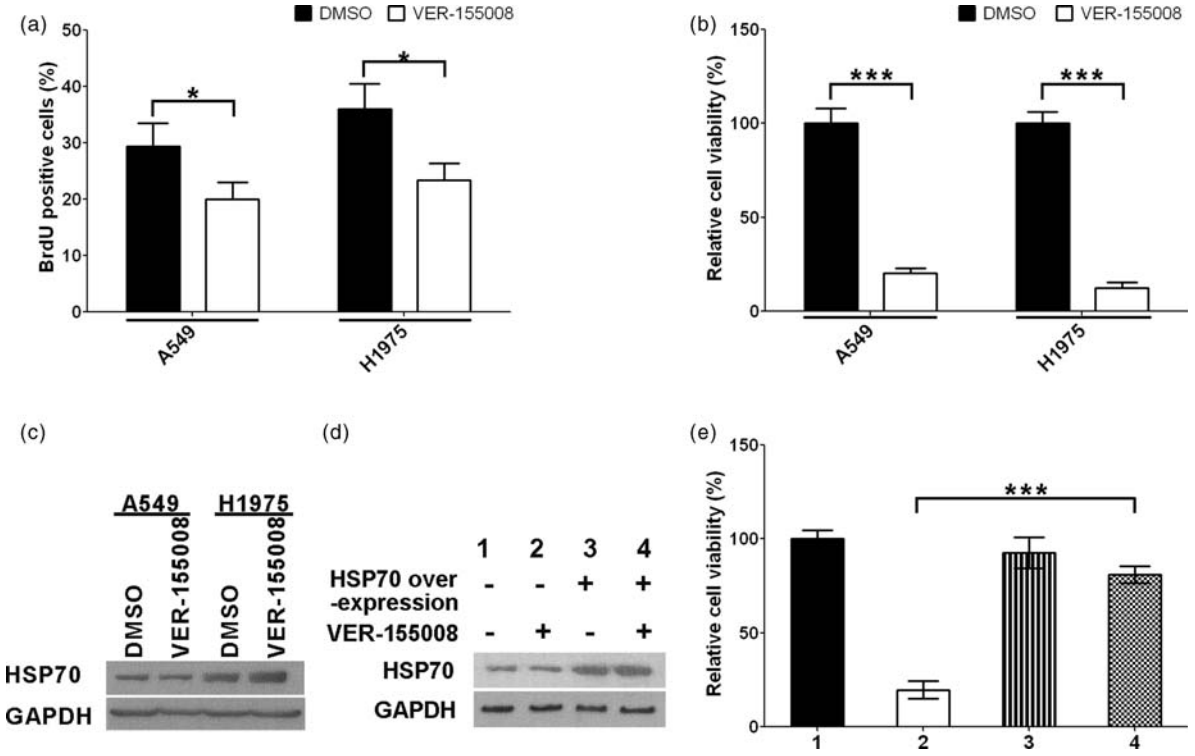

Figure 2 HSP70 inhibitor, VER-155008, inhibits proliferation of A549 and H1975 cells. (a) The percentages of BrdU-positive cells in A549 and H1975 cells treated with or without VER-155008. Results presented as Mean ± SD, $n = 3$. (b) Relative cell viabilities of A549 and H1975 cells treated with or without VER-155008. (c) The expression levels of HSP70 in A549 and H1975 cells treated with or without VER-155008. (d) The protein levels of HSP70 and GAPDH in A549 cells with indicated treatments. (e) Relative cell viabilities of above treated cells

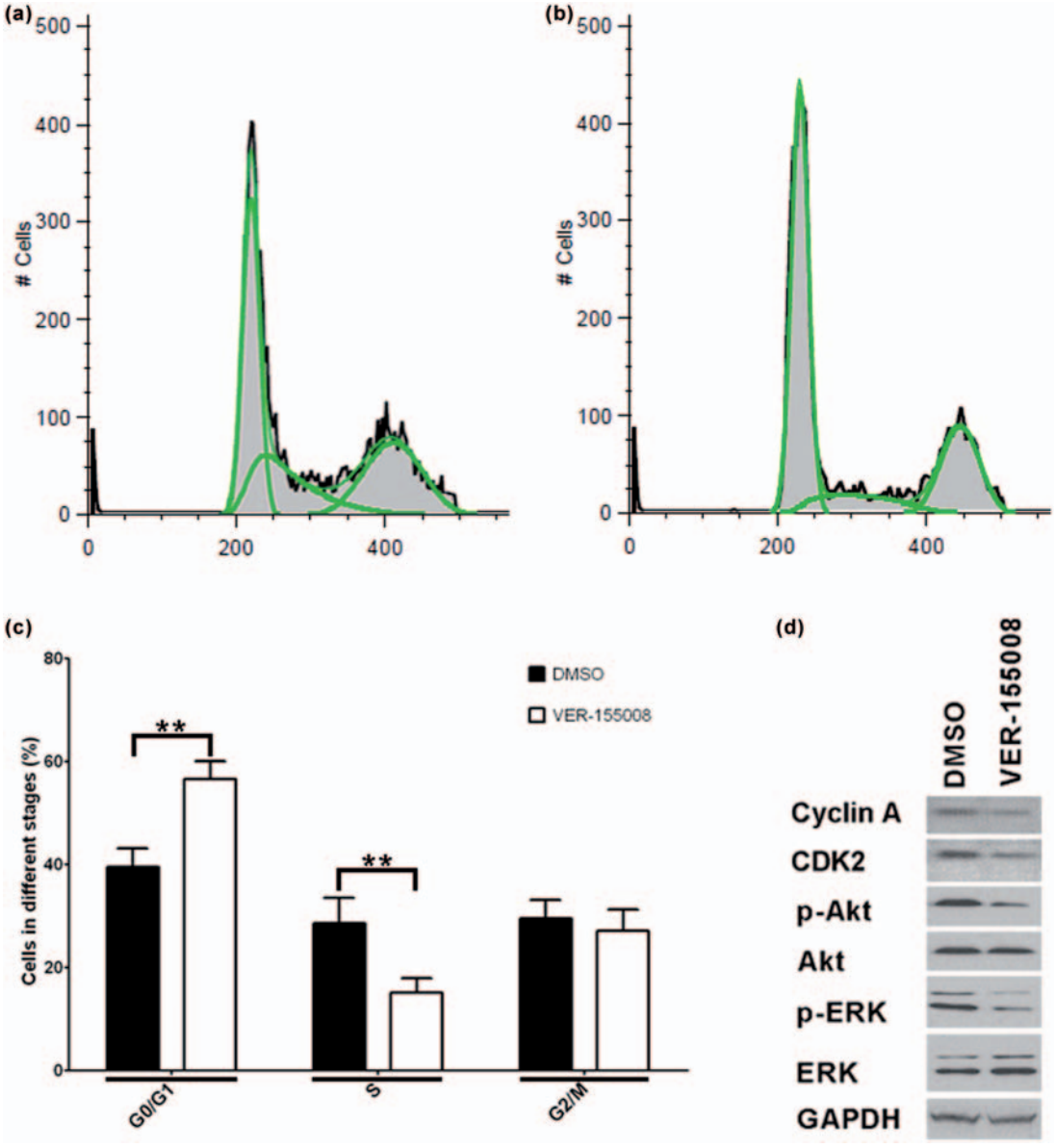

Figure 3 VER-155008 inhibits cell cycle progression of A549 cells. (a and b) Representative figures of FACS plots on A549 cells treated with or without VER-155008. (c) Quantification of FACS analysis, Mean ± SD, $n = 3$. (d) The protein expression levels of Cyclin A, CDK2, phosphorylated Akt, Akt, phosphorylated ERK, ERK in A549 cells treated with or without VER-155008. (A color version of this figure is available in the online journal.)

acquiring data on flow cytometer (BD FACSCalibur, BD Biosciences).

Ionization radiation

Cells (2×10^4) plated in dishes were treated with or without VER-155008 (5 μM) for two days, and then the cells were irradiated by using an X-ray irradiator (MBR-1505R2, Hitachi Medico, Japan) for indicated dosages. One percent of the cells were re-plated in a new dish. After seven days, the numbers of the focuses formed in dishes were counted. The surviving fraction (focus number of an irradiated group/that of the non-irradiated group) was calculated. In addition, the cells treated by 2 Gy radiation were analyzed by Guava Nexin assay performed following manufactory protocol (Millipore). The data were analyzed by using the software provided by the company.

Statistics

Experimental results are shown as the mean ± SD. Statistical analyses were performed by unpaired Students *t*-test or analysis of variance assuming unequal variance unless otherwise indicated using SigmaPlot 11.0 (San Jose, CA, USA). Significance was defined as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Results

Recent studies have demonstrated that HSP70 plays an important role in regulating NSCLC growth. However, a comprehensive search on PubMed revealed that there is

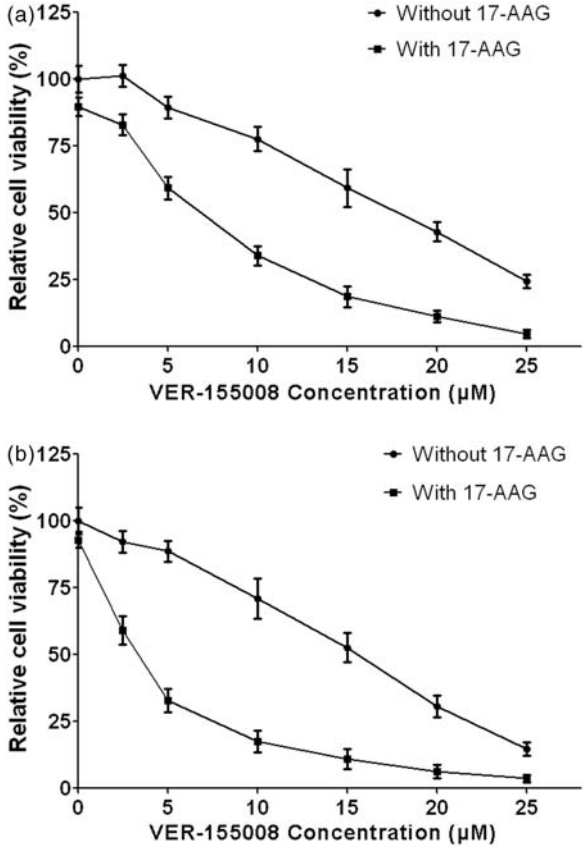

Figure 4 VER-155008 is more potent when combined with 17-AAG. (a) The dose-response curve of A549 cells to VER-155008, cells were simultaneously treated with or without 17-AAG (100 nM), Mean ± SD, $n = 3$. (b) Similar to (a), but with H1975 cells

a lack of data demonstrating proof-of-concept of HSP70 as a promising molecular target for NSCLC treatment. We first sought to test whether HSP70 protein is differentially expressed in NSCLC cell lines compared to normal counterparts. Results indicated that malignant NSCLC cell lines (A549 and H1975) exhibited higher HSP70 protein expression compared to a normal counterpart cell line (WI38) (Figure 1a). To further characterize the role of HSP70 in NSCLC tumor growth, we utilized shRNA-mediated knockdown of HSP70 in the above-mentioned NSCLC cell lines (Figure 1b). As expected, we found that HSP70 knockdown significantly inhibited cell proliferation as manifested by a reduction in percent of BrdU positive cells in both NSCLC cell lines (Figure 1c). Consistent with this observation, we also found that cell viability was significantly decreased after HSP70 knockdown in NSCLC cells (Figure 1d).

Next, we sought to understand/investigate whether small molecule inhibitors targeting HSP70 could reduce NSCLC cell growth. Results showed that VER-155008, a novel inhibitor of HSP70 that inhibits growth of myeloma and colon cancer cells, also inhibits cell proliferation and cell viability in both NSCLC cell lines tested (Figure 2a and b). Western blots indicated that HSP70 protein expression was unchanged after VER-155008 treatment, consistent with VER-155008 specifically interacting with the ATPase binding domain of HSP70, and inhibiting its activity (Figure 2c). In addition, we successfully overexpressed HSP70 in A549 cells (Figure 2d), and we observed that overexpression of HSP70 can largely, if not completely, rescue cells from inhibition by VER-155008. But interestingly, HSP70 overexpression itself cannot increase cell viability (Figure 2e).

We next sought to determine the mechanism by which VER-155008 inhibits NSCLC cell proliferation. Toward this goal, we preformed cell cycle analysis on cells treated with VER-155008 and observed a dramatic inhibition on cell cycle progression with treatment. Treatment with VER-155008 induced a significant increase of percentage of NSCLC cells in G_0/G_1 and dramatically decreases percentage of cells in S phase (Figure 3a–c). Notably, we did not find a significant change in percentage of cells in G_2/M phase (Figure 3c). Consistent with this observation, western blots for cell cycle progression markers such as cyclin A, CDK2, phosphorylated Akt, and ERK were also dramatically decreased following VER-155008 treatment (Figure 3d).

In addition, we tested whether there is any synergistic effect between 17-AAD, a well-known inhibitor for HSP90, and VER-155008 on NSCLC growth. MTT-based cell viability assays revealed that low concentration of 17-AAD (100 nM) dramatically increased VER-155008-mediated cell growth inhibition in both NSCLC cell lines (Figure 4a and b). This suggests that both HSP90 and HSP70 are involved in NSCLC cell growth.

Because several previous studies showed that HSP70 might have a protective role in cell subjected to IR, we hypothesized that inhibition of HSP70 would sensitize cancer cells to IR treatment. Indeed, when pretreated with 5 μM of VER-155008, A549 cells were much more vulnerable to IR (Figure 5a). Results also showed that IR induces cell death through apoptosis, and HSP70 inhibition can dramatically exaggerate pro-apoptotic effect of IR (Figure 5b–d).

Discussion

HSPs family members are among the most highly expressed cellular proteins.[23] HSPs were first identified from denatured cellular proteins that function to protect cells from heat damage. They account for 1–2% of total protein in unstressed cells, which increases to 4–6% of total protein under heat stress.[24]

Over the past decade, there has been a growing interest in roles of HSPs in cancer. The HSP70 subfamily of HSPs consists of molecular chaperones that are approximately 70 kDa in size and serve as critical regulators of homeostasis. These ATPases unfold misfolded or denatured proteins, protect nascently translating proteins, facilitate protein transportation, and reduce toxic protein aggregates. Generally, the HSP70 subfamily serves as a pivotal buffering system for a wide spectrum of extrinsic or intrinsic stimuli that are critical for cancer cell survival, and abnormal hyper-expression of HSP70 proteins is a marker for poor prognosis in cancers.[25] Similarly, HSP90 plays a similar role in protein folding, interacting with a different spectrum of client proteins. HSP90 docking proteins have been well characterized for their pivotal roles in cancer cell

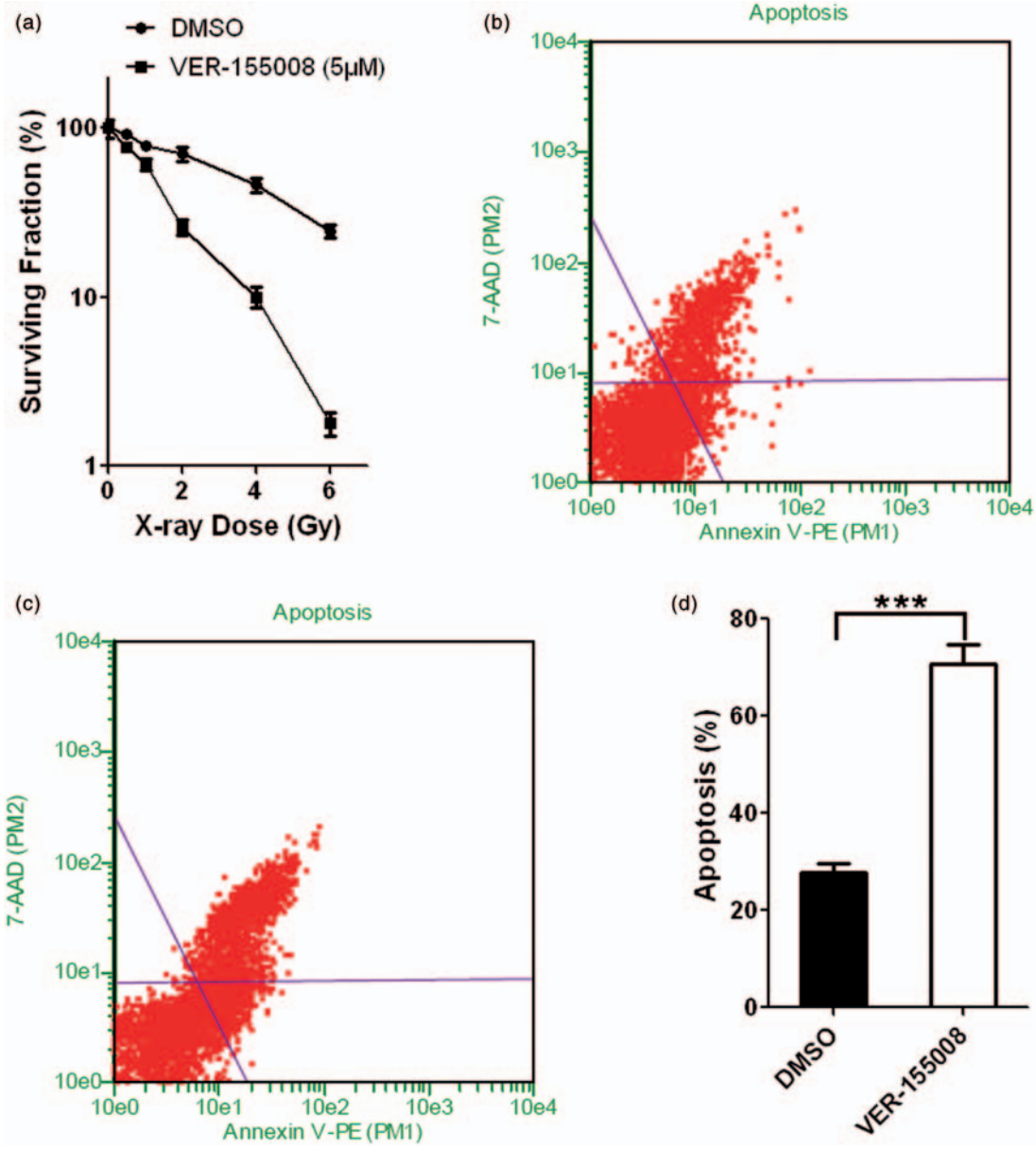

Figure 5 VER-155008 treatment sensitizes A549 cells to IR. (a) The surviving fractions of the cells with (5 μM) or without VER-155008 pretreatment after IR. (b) The representative picture of Guava Nexin assay on cells treated by 2 Gy IR. (c) The representative picture of Guava Nexin assay on cells treated by 2 Gy IR and 5 μM of VER-155008. (d) Quantification of Guava Nexin assay on cells (with or without VER-155008 pretreatment) treated by 2 Gy IR, Mean ± SD, $n = 3$. (A color version of this figure is available in the online journal.)

proliferation and tumor progression. Examples include growth factor receptors such as EGFR,[26] PI3 K, and Akt,[27] mutant proteins such as v-src, fusion oncogene Bcr/Abl, and mutant forms of p53.[28]

In the current study, we were interested in understanding whether HSP70 inhibitors exert strong antitumor activity in NSCLC given the abundant literatures indicating that HSP70 inhibitors can be used for the management of other solid tumors. In addition, we explored whether HSP70 inhibition combined with HSP90 inhibition exerts a potent synergistic effect on inhibition of NSCLC cell growth given the distinct docking partner protein spectrum of HSP70 and HSP90 family members despite the fact that HSP70 and HSP90 often coexist in the same complex regulating protein homeostasis. As expected, our results revealed that VER-155008, an HSP70-specific inhibitor, potently inhibits NSCLC cell growth and cell cycle progression. Interestingly, we also found that 17-AAG, an HSP90 inhibitor, and radiation exert potent synergistic effects with VER-155008 in NSCLC cell lines.

Consistent with our observations, other studies have shown a role for HSP70 and HSP90 in other malignancies. Fredly *et al.*[29] showed that serum HSP70 and HSP90 level is associated with all-trans retinoid acid and valproic acid treatment in acute myeloid leukemia patients. In addition,

expression of HSP70 and HSP90 are also associated with mammary gland neoplasm[30] and colorectal cancer.[31] We also found that there appears to be cross talk between HSP70 and HSP90 signaling machinery. As such, there is evidence showing that HSP90-dependent cancer cell survival is at least partially regulated by HSP70-dependent signaling,[32] and co-inhibition of HSP70 and HSP90 synergistically sensitizes nasopharyngeal carcinoma cells to thermotherapy.[33] However, there is lack of such data in lung cancer pathophysiology and our results provide novel insight and proof-of-principle showing the feasibility of managing NSCLC by targeting both HSP70/HSP90 signaling machinery. When combined with radiotherapy, we would expect robust synergistic effect, which is also observed in the previous mentioned data.

As for the future directions, it seems to be intriguing to identify the common downstream signaling pathways for HSP70 and HSP90 as the underlying mechanism for the above-mentioned observations. In various conditions, HSP70 and HSP90 seem to associate with each other in one complex via a common docking protein Hop or ST1P1.[34] Although there has been relatively lack of information on the role of Hop in tumor biology, previous results showed that elevated Hop protein expression is associated with human colon cancer,[35] hepatocellular carcinoma,[36] and pancreatic cancer.[37] Of note, there are a significant number of pro-oncogenic proteins which associate with HSP70/HSP90 complex and exert their positive regulatory effect on tumor biology.[38] Examples include, but not limited to, BAG1, BAG3, Aha1, p23, Cdc37, FKBP1, FKBP2, etc.[38] Although it is hard to pinpoint which docking protein is involved in the current scenario of VER-155008-induced NSCLC cell growth inhibition, it is reasonable to hypothesize that VER-155008 inhibits pro-oncogenic protein and/or facilitates tumor suppressor protein binding to this complex and this may partially explain the above-mentioned observations.

In conclusion, we identified that HSP70 governs NSCLC cell growth and cell cycle progression. Furthermore, VER-155008, a specific HSP70 inhibitor, exerts a potent inhibitory effect on NSCLC cell growth and robustly facilitates radiation-induced cancer cell death. Importantly, we also found that an HSP90 inhibitor exerts a potent synergistic effect on NSCLC cell growth when combined with HSP70 inhibitors, which is in line with previous findings on HSP70 and HSP90 in other malignancies. Future studies will be directed to experiments showing whether HSP70 and HSP90 inhibitors synergistically inhibit NSCLC growth *in vivo*. These data suggest that HSP70 and HSP90 inhibitors may be novel, promising candidates for the management of NSCLC.

Author contributions: WW and WL designed the current study, performed the experiments, and collected the data. YS and LC designed the whole project, provided scientific mentorship on this project, interpreted the data, and drafted the manuscript.

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REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;**127**:2893–917
2. Rigas JR. Taxane-platinum combinations in advanced non-small cell lung cancer: a review. *Oncologist* 2004;**9Suppl 2**: 16–23
3. Yang P. Epidemiology of lung cancer prognosis: quantity and quality of life. *Methods Mol Biol* 2009;**471**:469–86
4. Ravagnan L, Gurbuxani S, Susin SA, Maisse C, Daugas E, Zamzami N, Mak T, Jäättelä M, Penninger JM, Garrido C, Kroemer G. Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol* 2001;**3**:839–43
5. Jaattela M. Over-expression of hsp70 confers tumorigenicity to mouse fibrosarcoma cells. *Int J Cancer* 1995;**60**:689–93
6. Vargas-Roig LM, Fanelli MA, Lopez LA, Gago FE, Tello O, Aznar JC, Ciocca DR. Heat shock proteins and cell proliferation in human breast cancer biopsy samples. *Cancer Detect Prev* 1997;**21**:441–51
7. Koomagi R, Stammler G, Manegold C, Mattern J, Volm M. Expression of resistance-related proteins in tumoral and peritumoral tissues of patients with lung cancer. *Cancer Lett* 1996;**110**:129–36
8. Zimmermann M, Nickl S, Lambers C, Hacker S, Mitterbauer A, Hoetzenecker K, Rozsas A, Ostoros G, Laszlo V, Hofbauer H, Renyi-Vamos F, Klepetko W, Dome B, Ankersmit HJ. Discrimination of clinical stages in non-small cell lung cancer patients by serum HSP27 and HSP70: a multi-institutional case-control study. *Clin Chim Acta* 2012;**413**:1115–20
9. Nylandsted J, Rohde M, Brand K, Bastholm L, Elling F, Jaattela M. Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. *Proc Natl Acad Sci U S A* 2000;**97**:7871–6
10. Frese S, Schaper M, Kuster JR, Miescher D, Jaattela M, Buehler T, Schmid RA. Cell death induced by down-regulation of heat shock protein 70 in lung cancer cell lines is p53-independent and does not require DNA cleavage. *Journal Thorac Cardiovasc Surg* 2003;**126**:748–54
11. Sadekova S, Lehnert S, Chow TY. Induction of PBP74/mortalin/Grp75, a member of the hsp70 family, by low doses of ionizing radiation: a possible role in induced radioresistance. *International* J Radiat Biol 1997;**72**:653–60
12. Park SH, Lee SJ, Chung HY, Kim TH, Cho CK, Yoo SY, Lee YS. Inducible heat-shock protein 70 is involved in the radioadaptive response. *Radiat Res* 2000;**153**:318–26
13. Bases R. Clonogenicity of human leukemic cells protected from cell-lethal agents by heat shock protein 70. *Cell Stress Chaperones* 2005;**10**:37–45
14. Calini V, Urani C, Camatini M. Overexpression of HSP70 is induced by ionizing radiation in C3H 10T1/2 cells and protects from DNA damage. *Toxicol In Vitro* 2003;**17**:561–6
15. Bases R. Heat shock protein 70 enhanced deoxyribonucleic acid base excision repair in human leukemic cells after ionizing radiation. *Cell Stress Chaperones* 2006;**11**:240–9
16. Hunt CR, Dix DJ, Sharma GG, Pandita RK, Gupta A, Funk M, Pandita TK. Genomic instability and enhanced radiosensitivity in Hsp70.1- and Hsp70.3-deficient mice. *Mol Cell Biol* 2004;**24**:899–911
17. Niu P, Liu L, Gong Z, Tan H, Wang F, Yuan J, Feng Y, Wei Q, Tanguay RM, Wu T. Overexpressed heat shock protein 70 protects cells against DNA damage caused by ultraviolet C in a dose-dependent manner. *Cell Stress Chaperones* 2006;**11**:162–9
18. Massey AJ. ATPases as drug targets: insights from heat shock proteins 70 and 90. *J Med Chem* 2010;**53**:7280–6
19. Kaiser M, Kuhnl A, Reins J, Fischer S, Ortiz-Tanchez J, Schlee C, Mochmann LH, Heesch S, Benlasfer O, Hofmann WK, Thiel E,

Baldus CD. Antileukemic activity of the HSP70 inhibitor pifithrin-mu in acute leukemia. *Blood Cancer J* 2011;**1**:e28
20. Williamson DS, Borgognoni J, Clay A, Daniels Z, Dokurno P, Drysdale MJ, Foloppe N, Francis GL, Graham CJ, Howes R, Macias AT, Murray JB, Parsons R, Shaw T, Surgenor AE, Terry L, Wang Y, Wood M, Massey AJ. Novel adenosine-derived inhibitors of 70 kDa heat shock protein, discovered through structure-based design. *J Med Chem* 2009;**52**:1510–3
21. Chatterjee M, Andrulis M, Stuhmer T, Muller E, Hofmann C, Steinbrunn T, Heimberger T, Schraud H, Kressmann S, Einsele H, Bargou RC. The PI3K/Akt signalling pathway regulates the expression of Hsp70, which critically contributes to Hsp90-chaperone function and tumor cell survival in multiple myeloma. *Haematologica* 2012;**98**:1132–41
22. Massey AJ, Williamson DS, Browne H, Murray JB, Dokurno P, Shaw T, Macias AT, Daniels Z, Geoffroy S, Dopson M, Lavan P, Matassova N, Francis GL, Graham CJ, Parsons R, Wang Y, Padfield A, Comer M, Drysdale MJ, Wood M. A novel, small molecule inhibitor of Hsc70/Hsp70 potentiates Hsp90 inhibitor induced apoptosis in HCT116 colon carcinoma cells. *Cancer Chemother Pharmacol* 2010;**66**:535–45
23. Csermely P, Schnaider T, Soti C, Prohaszka Z, Nardai G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol Ther* 1998;**79**:129–68
24. Crevel G, Bates H, Huikeshoven H, Cotterill S. The Drosophila Dpit47 protein is a nuclear Hsp90 co-chaperone that interacts with DNA polymerase alpha. *J Cell Sci* 2001;**114**(Pt 11): 2015–25
25. Murphy ME. The HSP70 family and cancer. *Carcinogenesis* 2013;**34**:1181–8
26. Lurje G, Lenz HJ. EGFR signaling and drug discovery. *Oncology* 2009;**77**:400–10
27. Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. *J Biol Chem* 2002;**277**:39858–66
28. Calderwood SK, Khaleque MA, Sawyer DB, Ciocca DR. Heat shock proteins in cancer: chaperones of tumorigenesis. *Trends Biochem Sci* 2006;**31**:164–72
29. Fredly H, Reikvam H, Gjertsen BT, Bruserud O. Disease-stabilizing treatment with all-trans retinoic acid and valproic acid in acute myeloid leukemia: serum hsp70 and hsp90 levels and serum cytokine profiles are determined by the disease, patient age, and anti-leukemic treatment. *Am J Hematol* 2012;**87**:368–76
30. Badowska-Kozakiewicz AM, Malicka E. Immunohistochemical evaluation of expression of heat shock proteins HSP70 and HSP90 in mammary gland neoplasms in bitches. *Pol J Vet Sci* 2012;**15**:209–14
31. Ruckova E, Muller P, Nenutil R, Vojtesek B. Alterations of the Hsp70/Hsp90 chaperone and the HOP/CHIP co-chaperone system in cancer. *Cell Mol Biol Lett* 2012;**17**:446–58
32. Matokanovic M, Barisic K, Filipovic-Grcic J, Maysinger D. Hsp70 silencing with siRNA in nanocarriers enhances cancer cell death induced by the inhibitor of Hsp90. *Eur J Pharm Sci* 2013;**50**:149–58
33. Cui XB, Yu ZY, Wang W, Zheng YQ, Liu W, Li LX. Co-inhibition of HSP70/HSP90 synergistically sensitizes nasopharyngeal carcinoma cells to thermotherapy. *Integr Cancer Ther* 2012;**11**:61–7
34. Carrigan PE, Sikkink LA, Smith DF, Ramirez-Alvarado M. Domain:domain interactions within Hop, the Hsp70/Hsp90 organizing protein, are required for protein stability and structure. *Protein Sci* 2006;**15**:522–32
35. Kubota H, Yamamoto S, Itoh E, Abe Y, Nakamura A, Izumi Y, Okada H, Iida M, Nanjo H, Itoh H, Yamamoto Y. Increased expression of co-chaperone HOP with HSP90 and HSC70 and complex formation in human colonic carcinoma. *Cell Stress Chaperones* 2010;**15**:1003–11
36. Sun W, Xing B, Sun Y, Du X, Lu M, Hao C, Lu Z, Mi W, Wu S, Wei H, Gao X, Zhu Y, Jiang Y, Qian X, He F. Proteome analysis of hepatocellular carcinoma by two-dimensional difference gel electrophoresis: novel protein markers in hepatocellular carcinoma tissues. *Mol Cell Proteomics* 2007;**6**:1798–808
37. Walsh N, Larkin A, Swan N, Conlon K, Dowling P, McDermott R, Clynes M. RNAi knockdown of Hop (Hsp70/Hsp90 organising protein) decreases invasion via MMP-2 down regulation. *Cancer Lett* 2011;**306**:180–9
38. Calderwood SK. Molecular cochaperones: tumor growth and cancer treatment. *Scientifica* 2013;**2013**:217513

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