Biochemical and Biophysical Research Communications 450 (2014) 73–80

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SNX-25a, a novel Hsp90 inhibitor, inhibited human cancer growth more potently than 17-AAG

Shaoxiang Wang [a,b,c,1], Xiao Wang [c,1], Zhan Du [d,1], Yuting Liu [a], Dane Huang [a], Kai Zheng [c], Kaisheng Liu [a], Yi Zhang [a], Xueyun Zhong [b,*], Yifei Wang [a,*]

[a] Guangzhou Jinan Biomedicine Research and Development Center, National Engineering Research Center of Genetic Medicine, Jinan University, Guangzhou 510632, China
[b] Department of Pathology, Jinan University, Guangzhou 510632, China
[c] School of Medicine, Shenzhen University, Shenzhen 518060, China
[d] The Second Affiliated Hospital of Nanchang University, Nanchang 330006, China

ARTICLE INFO

Article history:
Received 11 May 2014
Available online 28 May 2014

Keywords:
Hsp90
SNX-25a
17-AAG
Apoptosis
Cell growth
Client proteins

ABSTRACT

17-Allylamino-17-demethoxygeldanamycin (17-AAG), a typical Hsp90 inhibitor derived from geldanamycin (GA), has entered Phase III clinical trials for cancer therapy. However, it has several significant limitations such as poor solubility, limited bioavailability and unacceptable hepatotoxicity. In this study, the anticancer activity and mechanism of SNX-25a, a novel Hsp90 inhibitor, was investigated comparing with that of 17-AAG. We showed that SNX-25a triggered growth inhibition more sensitively than 17-AAG against many human cancer cells, including K562, SW-620, A375, Hep-2, MCF-7, HepG2, HeLa, and A549 cell lines, especially at low concentrations (<1 μM). It showed low cytotoxicity in L-02, HDF and MRC5 normal human cells. Compared with 17-AAG, SNX-25a was more potent in arresting the cell cycle at G2 phase, and displayed more potent effects on human cancer cell apoptosis and Hsp90 client proteins. It also exhibited a stronger binding affinity to Hsp90 than 17-AAG using molecular docking. Considering the superiority effects on Hsp90 affinity, cell growth, cell cycle, apoptosis, and Hsp90 client proteins, SNX-25a is supposed as a potential anticancer agent that needs to be explored in detail.

1. Introduction

Heat shock protein 90 (Hsp90) is a molecular chaperone that is responsible for modulating the stability and activity of its client proteins [1]. The Hsp90 client proteins are almost exclusively signaling proteins like kinases and transcription factors, which play a critical role in regulating cell growth, cell cycle, survival, and apoptosis [2,3]. As compared to normal tissues, Hsp90 is overexpressed in solid tumors and hematological malignancies. Therefore, targeting of Hsp90 with chemical inhibitors may induce the degradation of oncogenic proteins and disrupt multiple oncogenic processes [4].

The ansamycin antibiotic geldanamycin (GA), as the first natural Hsp90 inhibitor, was found to bind to an ATP pocket in the N-terminal domain of the protein [5], which has led to the discovery of several other Hsp90 inhibitors currently undergoing clinical trials. 17-allylamino-17-demethoxygeldanamycin (17-AAG), an analogue of GA, is the first Hsp90 inhibitor entered into clinical trials to treat various types of cancers [6,7]. However, GA is too toxic for clinical use, while 17-AAG has several significant limitations, including poor solubility, limited bioavailability and unacceptable hepatotoxicity [7,8]. These drawbacks have catalyzed efforts to identify novel scaffolds with improved pharmacological properties for clinical applications.

A novel class of indol-4-one and indazol-4-one derived from 2-aminobenzamide that potently inhibit Hsp90 has been discovered [9]. Previous studies carried out in our lab have shown that SNX-2112, a 2-aminobenzamide Hsp90 inhibitor, exerts its growth inhibitory and apoptosis-inducing activity in many human cancer cells [10–13]. However its development has been discontinued recently because of ocular toxicity seen in animal models and in a separate phase I study [14]. Thus, design of a SNX-2112 alternative, based on the 2-aminobenzamide scaffold, might be a feasible way to meet the clinical needs.

SNX-25a, with a 2-(trans-4-hydroxy-cyclohexylamino) side chain, is a novel 2-aminobenzamide inhibitor of Hsp90 optimized by structure–activity relationship (SAR) explorations for high Hsp90 affinity [9]. To our knowledge, the anticancer effect of SNX-25a has not been reported. Here we tested the anticancer activities and mechanisms of SNX-25a on several human cancer

* Corresponding authors. Fax: +86 20 85223426.
E-mail addresses: tzxy@jnu.edu.cn (X. Zhong), twang-yf@163.com (Y. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.05.076
0006-291X/

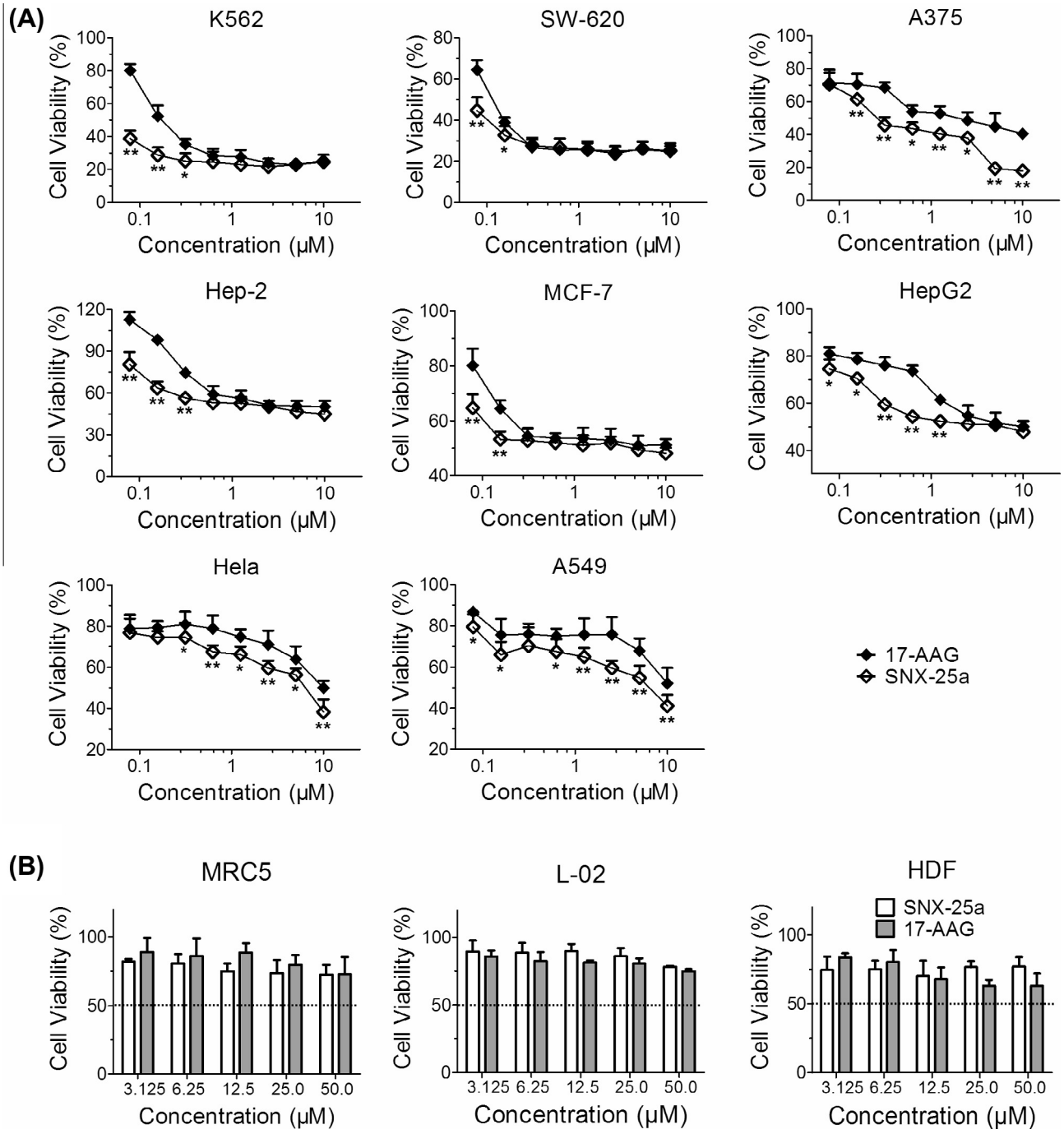

Fig. 1. Cytotoxic effects of SNX-25a and 17-AAG on human cancer cells (A) and normal cells (B) Cells were treated with various concentrations of SNX-25a and 17-AAG for 72 h. Cytotoxic effect of the compounds was determined by MTT assay. Percentages of viable cells were calculated by comparing treated and solvent control cells. Data are the mean ± S.D. of three replicates ($^{*}P < 0.05$; $^{**}P < 0.01$).

cells, compared with 17-AAG. Initially, the growth inhibitory effects of SNX-25a and 17-AAG on human cancer lines were investigated. To demonstrate more details, effects of the two compounds on cell cycle distribution, apoptotic rate, levels of Hsp90 client proteins, and Hsp90 affinity were also determined.

2. Materials and methods

2.1. Reagents and antibodies

SNX-25a was prepared in our laboratory with purities of >98.0% according to a previously described procedure [9]. 17-AAG was purchased from Sigma (Sigma, USA). SNX-25a and 17-AAG were dissolved in dimethyl sulfoxide (DMSO) at 10 mM stock solution and stored at −20 °C for *in vitro* experiments. Antibodies against IKKα, Raf-1, CHK1 and GSK3β were purchased from Epitomics (Burlingame, CA), and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was obtained from Millipore (Billerica, MA).

2.2. Cell culture

The normal human diploid fibroblast (HDF) was isolated from the foreskins of newborns using the procedure reported elsewhere

[15,16]. Other ten cell lines were from the Cell Bank of China Science Academy (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM), Minimum Essential Medium (MEM) or RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum.

2.3. MTT assay

Exponentially growing cells were seeded at 5–10 × 10^3 cells per well in 96-well culture plates for 24 h. Cells were exposed to increasing concentrations (0–10 μM) of SNX-25a or 17-AAG for 72 h. HDF, MRC5 and L-02 normal human cells were exposed to SNX-25a or 17-AAG at various concentrations (0–50 μM) for 72 h. The equal volume of DMSO was used as the solvent control. MTT solution (10 μl) was added to each well (0.5 mg/ml) and incubated for another 4 h. Light absorbance of the solution was measured at 570 nm, with a reference wavelength of 630 nm, on a multiscanner autoreader (M450; Bio-Rad, USA). The IC_{50} values were calculated using the PrismPad program.

2.4. Cell cycle analysis

To determine the effects of SNX-25a and 17-AAG on the cell cycle distribution, cancer cells were incubated with SNX-25a or 17-AAG (1.0 μM) for 48 h. Both suspended and adhered cells were collected and fixed in 70% ethanol overnight. Cells resuspended in PBS containing 50 μg/ml PI, 0.1 mg/ml RNase, and 5% Triton X-100 and incubated at 37 °C for 30 min. Cells were analyzed on a flow cytometer (Becton Dickinson, San Jose, CA), and the percentages of cells in different phases of the cell cycle were determined using Becton Dickinson CellQuest software.

2.5. Apoptosis assay

Cancer cells (1.5 × 10^6 cells/ml) were cultured with SNX-25a or 17-AAG (1.0 μM) for 48 h. Cells were harvested by trypsinization and resuspended in 500 μl of 1× binding buffer containing Annexin V-FITC and PI. Samples were incubated in the dark for 15 min at room temperature and analyzed by the flow cytometry.

2.6. Immunoblotting

K562 (15 × 10^6 cells/ml) cells were incubated with SNX-25a or 17-AAG (1.0 μM) for 0, 6, 12, 24 or 48 h. Cells were collected and washed with ice-cold PBS for three times. Then, cells were lysed with RIPA buffer for 30 min on ice. Total cellular protein (~25 μg) from each samples was loaded onto 8–12% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) gels. The proteins were transferred to polyvinylidene fluoride (PVDF) membrane (Millipore) and membranes were blocked with 5% non-fat milk for 1 h. The membranes were probed with primary antibodies overnight, washed with TBST for 30 min, exposed to horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h, and washed again in TBST for 30 min. Immunoreactive material was detected by using the chemiluminescence method. GAPDH was used as the loading control.

2.7. Docking assay

The affinity of SNX-25a and 17-AAG against Hsp90 was determined by MOE docking assay. Crystal structure of Hsp90 was taken from the Protein DataBank (PDB code: 3R92). SNX-25a and 17-AAG were converted to 3D structures, and energy was minimized in MOE. The binding site of Hsp90 was minimized using the AMBER 99 force field in MOE with the default parameter. The two compounds were docked, employing Triangle Matcher as the placement method and the function London dG as the first scoring function. The refinement was set to force field (AMBER 99), and the docked poses were energy minimized in the receptor pocket [17]. The conformations of lowest energy were given, and a lower scoring value indicated a more favorable binding.

2.8. Statistical analysis

Data were evaluated by the Welch t-test when only 2 value sets were compared. One-way ANOVA followed by Dunnett's test was used for comparisons of 3 or more groups. Results were expressed as means ± S.D. with significance at $^{*}P < 0.05$ or $^{**}P < 0.01$.

3. Results

3.1. SNX-25a inhibited cancer cell growth more potently than 17-AAG

We compared the growth inhibitory effects of SNX-25a and 17-AAG on human cancer lines originating from bone marrow, colon, skin, larynx, breast, liver, cervix, and lung. All cells were exposed to various concentrations (0–10 μM) for 72 h and cell viability was quantified by MTT assay. As shown in Fig. 1A, SNX-25a and 17-AAG significantly inhibited the growth of eight human cancer cell lines in a dose-dependent manner. The most two sensitive cell line were K562 and SW-620 (IC_{50}<0.01 μM). The IC_{50} values of other cancer cells were from 0.41 μM to 6.69 μM (Table 1). In all 8 cancer cell lines that we studied, SNX-25a was more potent than 17-AAG, especially at the low concentrations of <1 μM. For the remaining experiments, we adopted 1 μM as the optimal concentration.

3.2. SNX-25a exerted low cytotoxicity toward human normal cells

The cytotoxic effects of the two agents on three human normal cell lines (L-02, HDF, and MRC5 cells) were determined by MTT assay. The cells were exposed to SNX-25a or 17-AAG at concentrations from 3.13 to 50.0 μM for 72 h. There was no significant difference between SNX-25a and 17-AAG in the toxic effects of normal cells (Fig. 1B). The value of IC_{50} could not be determined even at the high concentration of 50.0 μM (Table 1), suggesting that SNX-25a exerted acceptable cytotoxicity toward normal cells and high selectivity in cancer cells.

Table 1
IC_{50} values of SNX-25a and 17-AAG.

Cell lines	Drug: IC50 (μM)	
	SNX-25a	17-AAG
Human cancer cells		
K562 (leukemia)	<0.01**	0.23 ± 0.11
SW-620 (colon carcinoma)	<0.01*	0.08 ± 0.07
A375 (melanoma)	0.41 ± 0.10**	2.09 ± 0.82
Hep-2 (laryngeal cancer)	2.45 ± 1.39*	4.20 ± 2.77
MCF-7 (breast cancer)	3.02 ± 1.80	4.61 ± 2.71
Hep-G2 (liver cancer)	3.66 ± 1.90*	7.02 ± 2.14
Hela (cervical cancer)	6.09 ± 3.11*	>10.00
A549 (lung cancer)	6.69 ± 3.32*	>10.00
Normal human cells		
MRC5 (fetal lung fibroblasts)	>100.00	>100.00
L-02 (liver cells)	>100.00	>100.00
HDF (dermal fibroblasts)	>100.00	>100.00

Cells were cultured with various concentrations (0–10 μM in cancer cells, 0–50 μM in normal cells) of compounds for 72 h. IC_{50} values of SNX-25a and 17-AAG towards eight cancer cell lines (K562, A375, MCF-7, Hep-2, HepG2, A549, SW620, and Hela) and three human normal cells (L-02, MRC5, and HDF) at 72 h as determined by using MTT assay. Data are the mean ± S.D. of three replicates.

* $P < 0.05$.

** $P < 0.01$.

3.3. SNX-25a induced cell cycle arrest at G2/M phase in cancer cells

To detect whether the growth-inhibitory effects of SNX-25a and 17-AAG was associated with the cell cycle arrest, we assessed their effects on the cell cycle distribution in five different cancer cell lines, including K562, Hep-2, A549, SW620 and Hela cells. Cells were treated with 1 μM SNX-25a or 17-AAG for 48 h, then subjected to flow cytometric analysis. We found that SNX-25a and 17-AAG induced G2 phase arrest in all five cancer cells in comparison to untreated cells (Fig. 2). Compared to 17-AAG, SNX-25a is more potent than 17-AAG in induction of cell cycle, suggesting that SNX-25a induced inhibitory effects may be mediated by cell cycle arrest.

3.4. SNX-25a was more potent than 17-AAG in inducing apoptosis of human cancer cells

To explore the mechanisms of cytotoxicity in cancer cells triggered by SNX-25a and 17-AAG, we performed apoptotic analysis in six cancer cells. The percentage of apoptotic cells increased after exposure to 1 μM SNX-25a and 17-AAG for 48 h, evaluated by flow cytometric analysis for Annexin-V/FITC and PI staining. SNX-25a and 17-AAG clearly induced apoptosis in all six cell lines (Fig. 3). SNX-25a at 1 μM induced apoptosis from 4.0% to 17.9% for K562; from 6.5% to 15.4% for SW-620; from 5.5% to 13.2% for Hep2; from 5.6% to 22.1% for Hela; and from 11.7% to 42.2% for A549, respectively. 8.8%–32.4% apoptotic cells were generated when these six

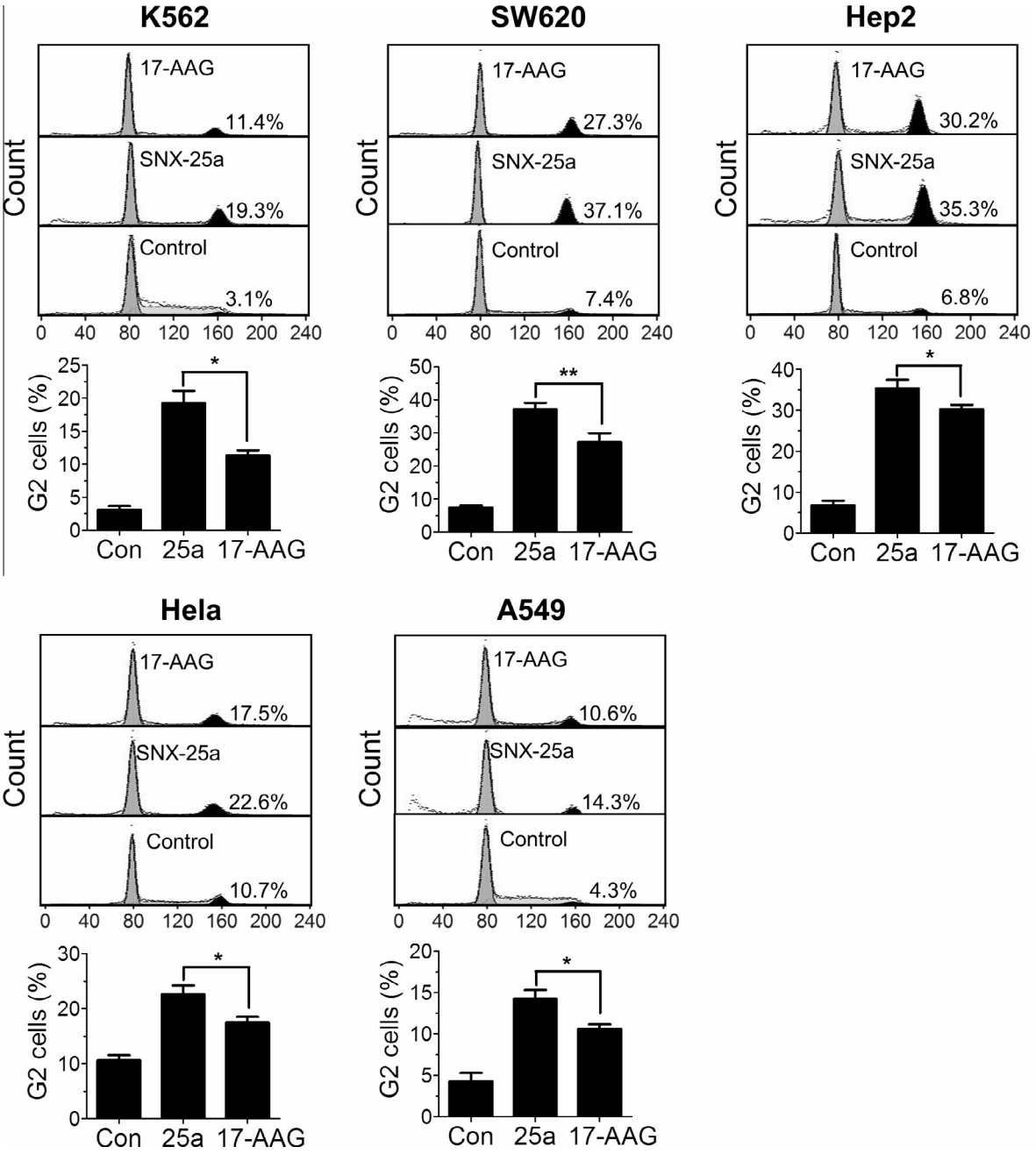

Fig. 2. Cell cycle analysis of cancer cells after treatment with SNX-25a and 17-AAG. Five cancer cells lines (K562, Hep-2, A549, SW620, and Hela cells) were used for cell cycle analysis. Cells were cultured with SNX-25a and 17-AAG at 1 μM, collected after 48 h and stained with PI. The DNA content and cell cycle distribution were analyzed by flow cytometry. Results represent mean ± SD of three replicates. Representative images from three experiments are shown, and percentages of cells in cycle arrest are indicated. Data are the mean ± S.D. of three replicates ($^{*}P < 0.05$; $^{**}P < 0.01$).

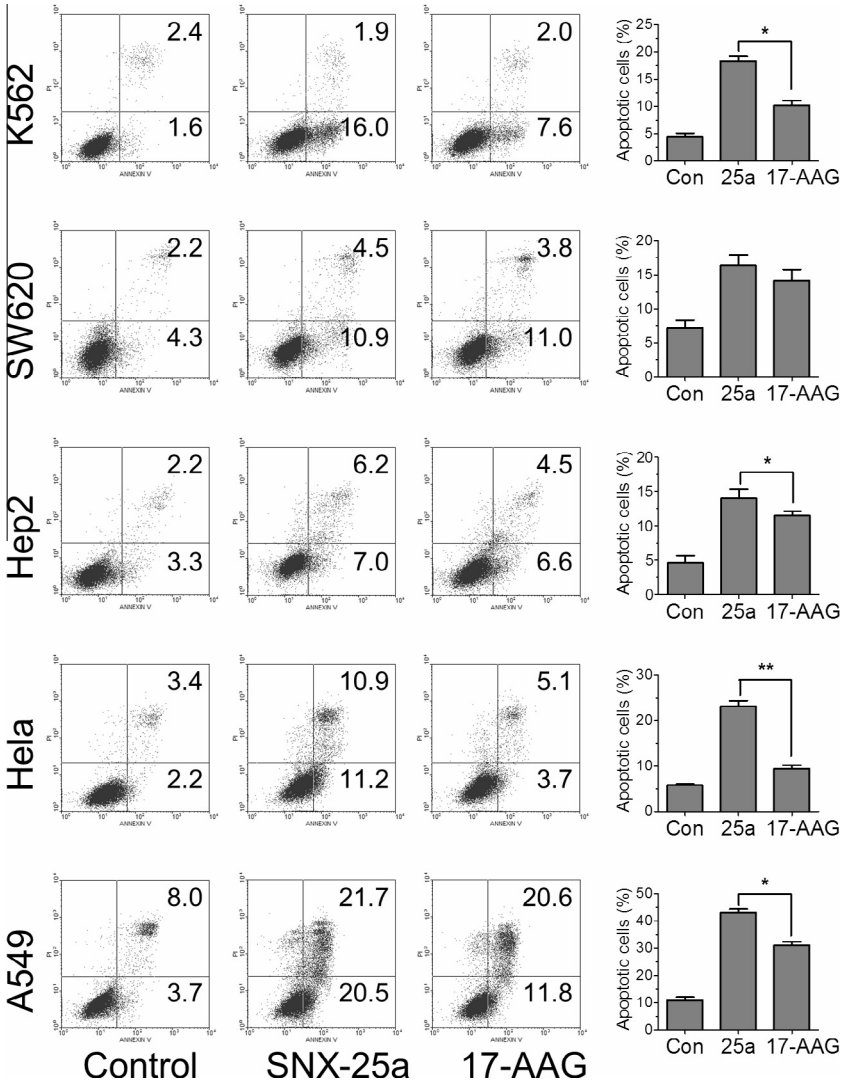

Fig. 3. Effects of SNX-25a and 17-AAG on induction of apoptosis in human cancer cells. K562, Hep-2, A549, SW620, and Hela cells were treated with control, SNX-25a and 17-AAG at 1 μM for 48 h. Apoptosis was detected by flow cytometric analysis based on PI (*Y*-axis) and Annexin V (*X*-axis) staining. Representative images from three experiments are shown, and percentages of apoptotic cells (lower and upper right-quadrants) are indicated. Data are the mean ± S.D. of three replicates ($^{*}P < 0.05$; $^{**}P < 0.01$).

cancer cell lines treated with 1 μM 17-AAG. Our results indicate that SNX-25a is more potent than 17-AAG in the apoptotic induction of human cancer cells.

3.5. SNX-25a down-regulated Hsp90 client proteins more potently than 17-AAG

Hsp90 inhibitors bind to the ATP pocket and suppress the ATPase activity of Hsp90, resulting in degradation of its client proteins [25]. To identify whether the Hsp90 client proteins are involved in the increased potency of SNX-25a compared with 17-AAG, Western blot was used to monitor the changes of the expression of four client proteins (including IKK, CHK1, GSK, and Raf) in K562 cells. As shown in Fig. 4A, these four Hsp90 client proteins were degraded in a time-dependent manner by SNX-25a and 17-AAG. Moreover, SNX-25a was more potent in suppressing Hsp90 client proteins than 17-AAG (Fig. 4B).

3.6. SNX-25a exhibited a stronger binding affinity to Hsp90 than 17-AAG

To compare the affinity to Hsp90 of SNX-25a and 17-AAG, docking studies were used to examine the fitness. A lower scoring value suggests a more favorable binding. It was clearly recognized that SNX-25a and 17-AAG interacted with Hsp90 binding pocket differently (Fig. 4C). We found a hydrogen bond residue (Phe-138) and a side chain donor molecule (Lys-58) contacted with SNX-25a. SNX-25a interacted with 18 residues in the pocket, whereas, 17-AAG only interacted with 11 residues, and major portion was exposed to the solvent. The scoring value of SNX-25a was −29.46 kcal/mol, which was lower than −21.4 kcal/mol in 17-AAG, indicating that SNX-25a had a better affinity to Hsp90 than 17-AAG.

4. Discussion

In this study, we revealed significant growth inhibitory effect of SNX-25a on multiple cancer cell types. This effect (1) is higher than 17-AAG; (2) seems to be generalized; (3) may be mediated by G2 phase arrest and cell apoptosis; and (4) may be provoked by the down-regulation of Hsp90 client proteins. In addition, we found that SNX-25a, a novel Hsp90 inhibitor, exerted higher affinity for Hsp90 than the typical Hsp90 inhibitor 17-AAG in molecular docking.

SNX-25a displayed better activity than 17-AAG in human cancer cells, with different sensitivity depended on specific cell types. One interpretation is that the expressions and functions of

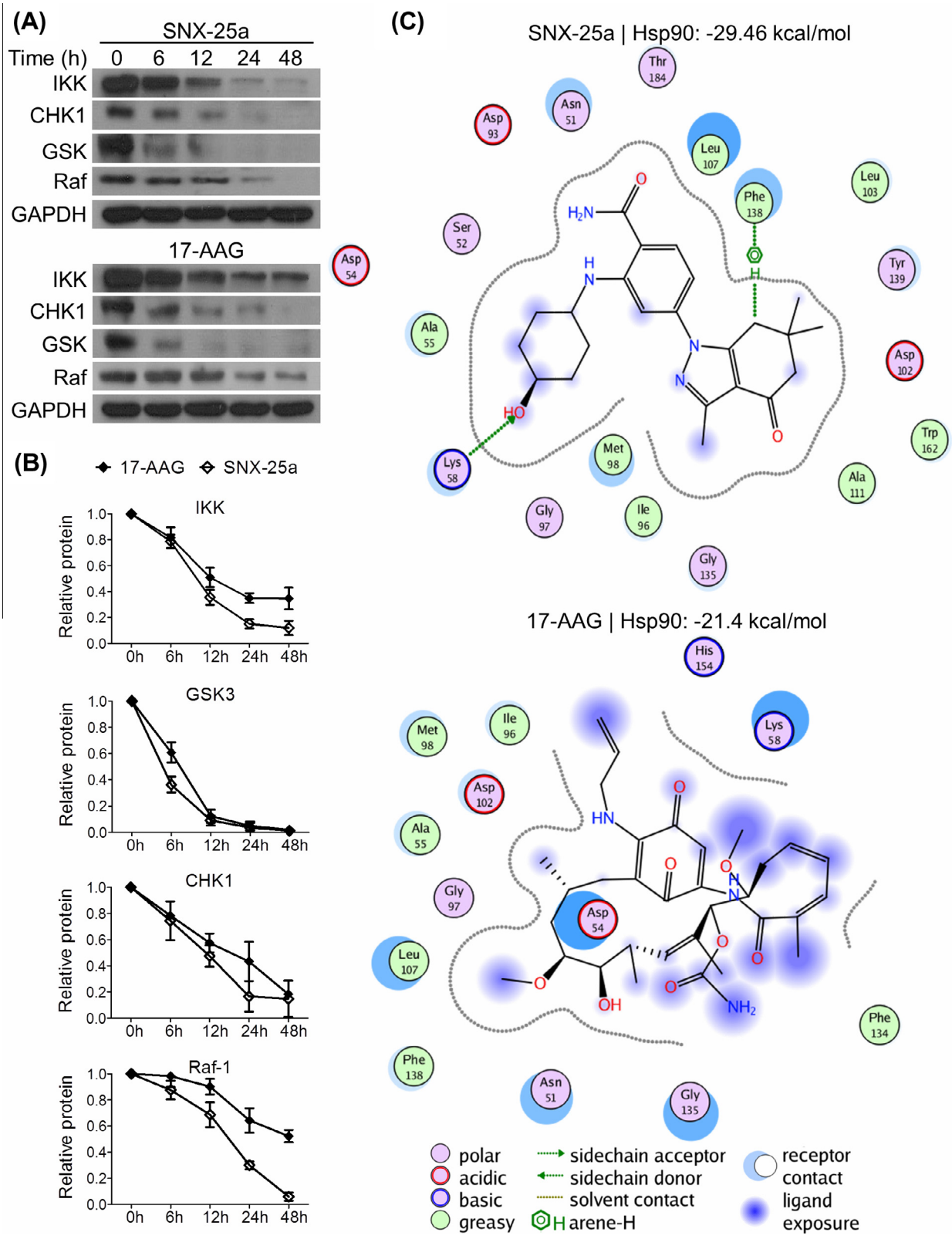

Fig. 4. Effects of SNX-25a and 17-AAG on Hsp90 client proteins and Hsp90 affinity. (A) Inhibition of Hsp90 client proteins by SNX-25a and 17-AAG. K562 cells were cultured with control (vehicle), 17-AAG and SNX-25a at 1 μM for the indicated times. Equal amounts of whole-cell lysates (25–50 μg) were analyzed by Western blot to detect the expressions of Hsp90 client proteins. (B) In the accompanying bar graphs, protein levels were normalized to GAPDH, which was used as a loading control. Results are shown as means ± S.D. of three independent experiments. (C) Docking of SNX-25a and 17-AAG into the N-terminal domain of Hsp90 (protein structure from PDB code 3R92). The conformations of lowest energy were shown, and a lower scoring value indicated a more favorable binding.

multiple Hsp90 isoforms are not uniform in different cell types. These Hsp90 isoforms include Hsp90α and Hsp90β in the cytoplasm and nucleus, GRP94 in the endoplasmic reticulum (ER), and TRAP1 in the mitochondria [18]. Hsp90α's functions include stress-induced cytoprotection and cell cycle regulation, whereas Hsp90β plays an important role in early embryonic development, signal transduction, and long term cell adaptation [19,20]. GRP94 is specialized for protein folding and plays a role in degradation of misfolded proteins through the ER-associated degradation pathway [21]. Meanwhile, TRAP1 is involved in signal transduction, protein folding, protein degradation, and morphologic evolution [22]. In fact, the development of Hsp90 inhibitors against specific Hsp90 isoforms may be a promising strategy for cancer therapy.

SNX-25a has strong selectivity in cell growth inhibition between cancer cells and normal cells. This is probably because the expression and molecular conformation of Hsp90 are greatly different in cancer and normal cells. In tumor cells, Hsp90 might be exclusively complexed with co-chaperones, forming a super-chaperone complex in a state of high affinity for ATP/ADP or ligands (e.g., Hsp90 inhibitors) of this regulatory pocket. However, in normal cells, Hsp90 is predominantly in a latent, uncomplexed, and low affinity state [23,24]. Therefore, compared with Hsp90 from normal cells, tumor Hsp90 is hypersensitive to Hsp90 inhibitors.

We show that SNX-25a displays the antitumor profile of the natural product Hsp90 inhibitors: growth inhibition, G2 cell cycle arrest, induction cell apoptosis, and degradation of Hsp90 clients [25]. Previously, we found that SNX-2112 can induce the degradation of Bcr-abl and Akt in K562 cells [26]. Here we focused on the levels of IKKα, GSK3, CHK1 and Raf-1, due to their critical roles in the proliferation of human leukemia cells. For instance, The IKK complex is a central regulator of NF-κB activation, and participates in up-regulation of several anti-apoptotic genes [27,28]. Holmes and Wang et al. have indicated that GSK3β can promote proliferation and/or survival in leukemia cells [29,30]. Emerging evidence suggests that CHK1 is a necessary regulator required for tumor growth and may contribute to anticancer therapy resistance [31,32]. The Ras/Raf/MEK/ERK signaling pathway has been shown to play a key role in the K562 cell survival [33,34]. Targeting Raf-1 gene expression by a DNA enzyme induces growth inhibition of leukemia cells [35]. Here we observed that SNX-25a induced time-dependent down-regulation of the expression of Hsp90 client proteins more potently than 17-AAG, which may be the mode of action of SNX-25a on induction of growth inhibition, cell cycle arrest, and apoptosis.

In conclusion, SNX-25a is a novel 2-aminobenzamide analogue of SNX-2112 with a higher efficacy than 17-AAG on inhibition of eight cancer cells growth. Consequently, this superiority effect merits further confirmation in xenograft experiments *in vivo*. The mode of action of antitumor activity may be associated with the induction of cell cycle arrest, apoptosis and Hsp90 client proteins degradation. Our results suggest that the novel Hsp90 inhibitor SNX-25a may represent a promising alternative to 17-AAG for clinical applications.

5. Conflict of interest

The authors have no conflict of interests to declare.

Acknowledgments

We thank Prof. Bao-Jian Wu from Jinan University (Guangzhou, China) for proofreading the manuscript.

This work was supported by grants from the National Natural Science Foundation of China (Grant 81201727), the China Postdoctoral Science Foundation (Grants 2012M511882, 2013T60827), the open project of State Key Laboratory of Molecular Oncology (Grant SKL-KF-2013-14), Guangdong Province and Ministry of Education Ministry of Science and Technology Products Research Combined Platform Project (Grant 2010B091000013), Science and Technology Project of Guangzhou City (Grant 2014J4100103), and the Natural Science Foundation of Guangdong Province (Grant S2012040006873).

Reference

[1] L.H. Pearl, C. Prodromou, Structure and mechanism of the Hsp90 molecular chaperone machinery, Annu. Rev. Biochem. 75 (2006) 271–294.
[2] J. Trepel, M. Mollapour, G. Giaccone, L. Neckers, Targeting the dynamic HSP90 complex in cancer, Nat. Rev. Cancer 10 (2010) 537–549.
[3] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[4] D. Mahalingam, R. Swords, J.S. Carew, S.T. Nawrocki, K. Bhalla, F.J. Giles, Targeting HSP90 for cancer therapy, Br. J. Cancer 100 (2009) 1523–1529.
[5] J.P. Grenert, W.P. Sullivan, P. Fadden, T.A. Haystead, J. Clark, E. Mimnaugh, H. Krutzsch, H.J. Ochel, T.W. Schulte, E. Sausville, L.M. Neckers, D.O. Toft, The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation, J. Biol. Chem. 272 (1997) 23843–23850.
[6] S.Z. Usmani, R. Bona, Z. Li, 17 AAG for HSP90 inhibition in cancer – from bench to bedside, Curr. Mol. Med. 9 (2009) 654–664.
[7] J.R. Porter, C.C. Fritz, K.M. Depew, Discovery and development of Hsp90 inhibitors: a promising pathway for cancer therapy, Curr. Opin. Chem. Biol. 14 (2010) 412–420.
[8] R.L. Cysyk, R.J. Parker, J.J. Barchi Jr., P.S. Steeg, N.R. Hartman, J.M. Strong, Reaction of geldanamycin and C17-substituted analogues with glutathione: product identifications and pharmacological implications, Chem. Res. Toxicol. 19 (2006) 376–381.
[9] K.H. Huang, J.M. Veal, R.P. Fadden, J.W. Rice, J. Eaves, J.P. Strachan, A.F. Barabasz, B.E. Foley, T.E. Barta, W. Ma, M.A. Silinski, M. Hu, J.M. Partridge, A. Scott, L.G. DuBois, T. Freed, P.M. Steed, A.J. Ommen, E.D. Smith, P.F. Hughes, A.R. Woodward, G.J. Hanson, W.S. McCall, C.J. Markworth, L. Hinkley, M. Jenks, L. Geng, M. Lewis, J. Otto, B. Pronk, K. Verleysen, S.E. Hall, Discovery of novel 2-aminobenzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents, J. Med. Chem. 52 (2009) 4288–4305.
[10] D.C. Chinn, W.S. Holland, J.M. Yoon, T. Zwerdling, P.C. Mack, Anti-tumor activity of the HSP90 inhibitor SNX-2112 in pediatric cancer cell lines, Pediatr. Blood Cancer 58 (2012) 885–890.
[11] M. Taipale, D.F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: emerging mechanistic insights, Nat. Rev. Mol. Cell Biol. 11 (2010) 515–528.
[12] T. Bachleitner-Hofmann, M.Y. Sun, C.T. Chen, D. Liska, Z. Zeng, A. Viale, A.B. Olshen, M. Mittlboeck, J.G. Christensen, N. Rosen, D.B. Solit, M.R. Weiser, 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, Clin. Cancer Res. 17 (2011) 122–133.
[13] H.A. Crissman, J.A. Steinkamp, Rapid, simultaneous measurement of DNA, protein, and cell volume in single cells from large mammalian cell populations, J. Cell Biol. 59 (1973) 766–771.
[14] A. Rajan, R.J. Kelly, J.B. Trepel, Y.S. Kim, S.V. Alarcon, S. Kummar, M. Gutierrez, S. Crandon, W.M. Zein, L. Jain, B. Mannargudi, W.D. Figg, B.E. Houk, M. Shnaidman, N. Brega, G. Giaccone, A phase I study of PF-04929113 (SNX-5422), an orally bioavailable heat shock protein 90 inhibitor, in patients with refractory solid tumor malignancies and lymphomas, Clin. Cancer Res. 17 (2011) 6831–6839.
[15] Y.H. Lee, N.H. Lee, G. Bhattarai, J.S. Yun, T.I. Kim, E.C. Jhee, H.K. Yi, PPARgamma inhibits inflammatory reaction in oxidative stress induced human diploid fibloblast, Cell Biochem. Funct. 28 (2010) 490–496.
[16] W.J. Bettger, S.T. Boyce, B.J. Walthall, R.G. Ham, Rapid clonal growth and serial passage of human diploid fibroblasts in a lipid-enriched synthetic medium supplemented with epidermal growth factor, insulin, and dexamethasone, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 5588–5592.
[17] D. Huang, Q. Gu, H. Ge, J. Ye, N.K. Salam, A. Hagler, H. Chen, J. Xu, On the value of homology models for virtual screening: discovering hCXCR3 antagonists by pharmacophore-based and structure-based approaches, J. Chem. Inf. Model. 52 (2012) 1356–1366.
[18] T. Kawano, T. Kobayakawa, Y. Fukuma, H. Yukitake, Y. Kikuchi, M. Shoji, K. Nakayama, A. Mizuno, T. Takagi, T.K. Nemoto, A comprehensive study on the immunological reactivity of the Hsp90 molecular chaperone, J. Biochem. 136 (2004) 711–722.
[19] A.S. Sreedhar, E. Kalmar, P. Csermely, Y.F. Shen, Hsp90 isoforms: functions, expression and clinical importance, FEBS Lett. 562 (2004) 11–15.
[20] D.S. Hong, U. Banerji, B. Tavana, G.C. George, J. Aaron, R. Kurzrock, Targeting the molecular chaperone heat shock protein 90 (HSP90): lessons learned and future directions, Cancer Treat. Rev. 39 (2013) 375–387.
[21] M. Marzec, D. Eletto, Y. Argon, GRP94: an HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum, Biochim. Biophys. Acta 2012 (1823) 774–787.

[22] D.S. Matassa, M.R. Amoroso, F. Maddalena, M. Landriscina, F. Esposito, New insights into TRAP1 pathway, Am. J. Cancer Res. 2 (2012) 235–248.
[23] A. Kamal, L. Thao, J. Sensintaffar, L. Zhang, M.F. Boehm, L.C. Fritz, F.J. Burrows, A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors, Nature 425 (2003) 407–410.
[24] M. Vilenchik, D. Solit, A. Basso, H. Huezo, B. Lucas, H. He, N. Rosen, C. Spampinato, P. Modrich, G. Chiosis, Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90, Chem. Biol. 11 (2004) 787–797.
[25] Y. Miyata, Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents, Curr. Pharm. Des. 11 (2005) 1131–1138.
[26] L. Jin, C.L. Xiao, C.H. Lu, M. Xia, G.W. Xing, S. Xiong, Q.Y. Liu, H. Liu, Y.C. Li, F. Ge, Q.D. Wang, Q.Y. He, Y.F. Wang, Transcriptomic and proteomic approach to studying SNX-2112-induced K562 cells apoptosis and anti-leukemia activity in K562-NOD/SCID mice, FEBS Lett. 583 (2009) 1859–1866.
[27] M. Karin, Nuclear factor-kappaB in cancer development and progression, Nature 441 (2006) 431–436.
[28] A. Israel, The IKK complex, a central regulator of NF-kappaB activation, Cold Spring Harb. Perspect. Biol. 2 (2010) a000158.
[29] T. Holmes, T.A. O'Brien, R. Knight, R. Lindeman, S. Shen, E. Song, G. Symonds, A. Dolnikov, Glycogen synthase kinase-3beta inhibition preserves hematopoietic stem cell activity and inhibits leukemic cell growth, Stem Cells 26 (2008) 1288–1297.
[30] Z. Wang, K.S. Smith, M. Murphy, O. Piloto, T.C. Somervaille, M.L. Cleary, Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy, Nature 455 (2008) 1205–1209.
[31] C. Cavelier, C. Didier, N. Prade, V. Mansat-De, V. Mansat-De Mas, S. Manenti, C. Recher, C. Demur, B. Ducommun, Constitutive activation of the DNA damage signaling pathway in acute myeloid leukemia with complex karyotype: potential importance for checkpoint targeting therapy, Cancer Res. 69 (2009) 8652–8661.
[32] J.H. Lee, M.L. Choy, L. Ngo, G. Venta-Perez, P.A. Marks, Role of checkpoint kinase 1 (Chk1) in the mechanisms of resistance to histone deacetylase inhibitors, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 19629–19634.
[33] L.S. Steelman, R.A. Franklin, S.L. Abrams, W. Chappell, C.R. Kempf, J. Basecke, F. Stivala, M. Donia, P. Fagone, F. Nicoletti, M. Libra, P. Ruvolo, V. Ruvolo, C. Evangelisti, A.M. Martelli, J.A. McCubrey, Roles of the Ras/Raf/MEK/ERK pathway in leukemia therapy, Leukemia 25 (2011) 1080–1094.
[34] P. Sancho, E. Galeano, M.C. Estan, I. Ganan-Gomez, C. Boyano-Adanez, Mdel, A.I. Garcia-Perez, Raf/MEK/ERK signaling inhibition enhances the ability of dequalinium to induce apoptosis in the human leukemic cell line K562, Exp. Biol. Med. (Maywood) 237 (2012) 933–942.
[35] J.M. Davis, P.M. Navolanic, C.R. Weinstein-Oppenheimer, L.S. Steelman, W. Hu, M. Konopleva, M.V. Blagosklonny, J.A. McCubrey, Raf-1 and Bcl-2 induce distinct and common pathways that contribute to breast cancer drug resistance, Clin. Cancer Res. 9 (2003) 1161–1170.