ORIGINAL ARTICLE

Synergistic antitumor activity of pro-apoptotic agent PAC-1 with cisplatinum by the activation of CASP3 in pulmonary adenocarcinoma cell line H1299

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Abstract

Aim: Evasion of apoptosis is a hallmark of human cancer cells. We sought to explore the potential synergistic antitumor activity and underlying mechanisms of the pro-apoptotic agent PAC-1 plus cisplatinum (Cis) in non-small cell lung cancer (NSCLC) cell lines.

Methods: The adenocarcinoma cell lines H1299, A549, PC9, H1650 and H1975 were used as in vitro models. Colorimetric MTT assays, Western blotting and flow cytometry were used to evaluate the anti-growth effects of PAC-1 and/or Cis and apoptosis status. The activated form of CASP3 (C-CASP3) was assessed by immunofluorescent staining.

Results: Single-agent Cis and PAC-1 were able to inhibit the cancer cell growth in certain dose ranges, with IC50 values of 1.9–11.7 and 5.6–14.8 μM, respectively. Sequential Cis→PAC-1 or concurrent Cis + PAC-1, but not PAC-1→Cis combinations showed synergistic effects on cell growth inhibition in H1299 cells (combination index, CI ≤ 0.6). In contrast, other combination modes mostly showed seemingly antagonistic effects (CI > 1.0). Flow cytometric analysis showed that Cis→PAC-1 sequential combination showed strong pro-apoptotic effects in H1299 cells. Western blots showed that in H1299, PC9 and H1975 cells, PAC-1 promoted the C-CASP3, but only in H1299 cells was there a synergistic effect with Cis on the CASP3 activation.

Conclusions: PAC-1 showed anti-tumor activity in NSCLCs in vitro and a synergistic effect with cisplatin in EGFRwtKRASwt H1299 cells. Our data suggest a potential treatment approach using cisplatin plus a pro-apoptotic agent acting via CASP3 activation for this subgroup of pulmonary adenocarcinomas.

Key words: apoptosis, CASP3, non-small cell lung cancer, PAC-1.

INTRODUCTION

Lung cancer is a leading cause of cancer-related deaths, worldwide and in China.1-4 Non-small cell lung cancer (NSCLC) is the major type, accounting for ~80% of lung cancers. Presently, for advanced disease, chemotherapy remains the cornerstone for multiple-modality management of this malignancy. Small-molecule inhibitors are also effective and serve as the standard for care in EGFR mutant5,6 or ALK-rearranged7 advanced lung cancers. Molecular-targeted therapies could be of benefit in specific molecular subgroups of patients, highlighting the importance of molecular subtyping for lung cancers. Temporary actionable molecular targets include EGFR,
ALK, ROS1, PD-1 and PD-L1. Additionally, there is still a high proportion of lung cancers with unknown targets. The present standard of care for these patients in the first-line setting is a platinum-based doublet chemotherapeutic regimen; however, the clinical response is only ~30%. Thus, methods to improve efficacy in patient subpopulations are of great interest. Novel approaches and therapeutics, in addition to EGFR and ALK TKIs, are needed to treat NSCLC.

“Resisting cell death” is a hallmark of malignant cancer cells. Molecules in the apoptotic pathway can be classified as regulators and effectors, and some may play key roles in the process of cancer development and evolution. Promotion of the pro-apoptotic activation process is one strategy for cancer treatment.

In this study, cell models with different genetic alterations, such as EGFR and KRAS mutations and ALK gene rearrangements, were used to evaluate the anti-tumor activity of the caspase 3 activator “procaspase-activating compound 1” (PAC-1) and its potential synergistic anti-cancer effects when combined with cisplatinum (Cis). We also sought to determine the underlying molecular mechanisms.

MATERIALS AND METHODS

Drugs and reagents

The pro-apoptotic agent PAC-1 was purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and dissolved in dimethyl sulfoxide as a 5.1 mmol/L stock solution. Cis was purchased from Jiangsu Haosen Pharmaceutical Ltd (Lianyungang, Jiangsu, China). The stock solutions of these drugs were diluted with culture medium before use. Antibodies against CASP3 (Caspase 3), C-CASP3 (cleaved Caspase 3), were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Rabbit anti-human CASP8 (Caspase 8), CASP9 (Caspase 9), XIAP, BCL-2 and ACTB (β-actin) antibodies were purchased from Abcam (Cambridge, MA, USA).

Cell lines

Human lung adenocarcinoma H1299 cells (homozygous TP53 partial deletion) and A549 cells (K-RAS exon 2 G12S) were purchased from the American Type Culture Collection (Manassas, VA, USA). Human lung adenocarcinoma cells H1650 (EGFR exon 19 delE746-A750), H1975 (EGFR exon 20 T790M + exon 21 L858R) and PC-9 (EGFR exon 19 delE746-A750) were kindly provided by Prof. Yilong Wu (Guangdong General Hospital). Cells were grown in RPMI 1640 medium, supplemented with 10% FBS plus penicillin (100 UI/mL) and streptomycin (100 μg/mL), at 37°C in a humidified atmosphere of 5% CO2.

METHODS

Evaluation of the anti-proliferative effects of PAC-1 or in combination with Cis

Cell viability was determined using the MTT assay following the method of Mosmann and Carmichael. The numbers of cells per well were 3000 for H1299 cells, 7000 for H1650 cells, 4000 for H1975 cells, 4000 for A549 cells and 3000 for PC-9 cells according to a previous pilot experiment to achieve OD values ranging from 0.8 to 1.2 after 72 h of culture. The IC50 value was the concentration resulting in 50% inhibition of cell growth after exposure to the drug compared with untreated control cells. Serial dilutions of Cis and PAC-1 were used in the cell cultures, and after 72 h of incubation, the ODs were measured using a spectrometer at 570 nm. IC25, IC50 and IC75 values were calculated using CompuSyn software (CompuSyn, Inc., Paramus, NJ, USA).

To evaluate the anti-proliferative effects of the combined treatments, cells were treated with three different treatment sequences: (i) pretreatment with Cis for 24 h, aspiration, one wash with PBS, followed by PAC-1 treatment for 48 h; (ii) pretreatment with PAC-1 for 48 h, aspiration, one wash with PBS, followed by Cis treatment for 24 h; and (iii) concomitant treatment with Cis and PAC-1 for 48 h followed by incubation in drug-free medium for 24 h. The two drugs were combined at a range of doses covering the IC25 through IC75 values of each drug in all cell lines. These serial doses were 0.11, 0.33, 1.0, 3.0, 9.0 and 27.0 μmol/L for Cis and 0.33, 1.0, 3.0, 9.0, 27.0 and 81.0 μmol/L for PAC-1 to calculate the combination index (CI) value. Constant ratio combination of Cis and PAC at molarity ratio 1:3 was used for tumor cell growth inhibition experiments. The concentration ranges of PAC-1 used on PC9 cells were physiologically achievable.

The results of sequential treatment with Cis and PAC-1 were analyzed according to the method of Chou and Talalay. The resulting CI value is a quantitative measure of the degree of interaction between different drugs, with CI values >1.1, 0.9–1.1 and <0.9 indicating antagonistic, additive and synergistic effects, respectively. The CI value was calculated using CompuSyn software. The formula for CI calculation was CI = (D1/(Dx)1 + (D2/(Dx)2, where (D1 and (D2 are the combined doses of each drug and Dx = Dm
Synergistic activity of PAC-1 and cisplatin

Western blot analysis of apoptotic pathway protein expression

To assess changes in apoptosis-related molecules in the H1299, H1975 and PC-9 cell lines by Western blotting, protein extracts were obtained from each experimental group (Cis, PAC-1, Cis followed by PAC-1, PAC-1 followed by Cis and concurrent Cis plus PAC-1) and controls. All doses of drug treatment were at IC50 of Cis or PAC-1; treatment time was the same as aforementioned in experiments of anti-proliferation effects of cell growth. Proteins from each cell line of each experimental group treated with the IC50 value of PAC-1 for 0, 3, 6, 12, 24 and 48 h were also isolated for analysis. Briefly, cells were collected and lysis solution supplemented with the proteinase inhibitor PMSF was added for 20 min at 4°C and the reaction mixtures were centrifuged (14 000 rpm). Supernatants were boiled in SDS buffer and quantified using the bicinchoninic acid assay method. Equal amounts of proteins and 8 μL of a marker were loaded on a 10% stacking gel for electrophoresis for 30 min at 60 V, then a further 60 min at 110 V electrophoresis in the separating gel, then transferred to a PVDF membrane.

Skimmed milk powder was used to block the nonspecific protein targets. Certain dilutions of specific primary antibodies were added for overnight incubation at 4°C. The primary antibodies were anti-β-actin (clone 13E5, #4970, CST) at 1:1000, anti-CASP3 (Clone 8G10, #9665, CST) at 1:1000, anti-C-CASP3 (Clone 5A1E, #9664, Cell Signaling, USA) at 1:1000, anti-XIAP (clone 3B6, #2045, CST) at 1:1000, anti-CASP9 (clone E23, ab32539, Abcam) at 1:500 for incubation. After the primary antibodies were added for overnight incubation, secondary antibodies were added for incubation overnight. Secondary antibody labeled by Alexa Fluor 647 labeled was added at room temperature for 1 h, followed by incubation with 100 μL Hoechst 33342 for 5 min. Finally, the wells were covered with a coverslip and observed under a confocal microscope (Leica). C-CASP3-positive cell rates were calculated by counting at least 100 cells under confocal microscopy.

Statistical analysis

Variables are reported as means ± standard errors or deviations from three representative independent experiments. The CI values of the two drugs for antiproliferative effects were calculated using CompuSyn software (ComboSyn, Inc.). Differences between groups in the apoptosis and cell cycle analyses were assessed by analysis of variance and post hoc LSD (Least Significant Difference) comparison tests using SPSS software. C-CASP3 immunostaining was compared by t-test between groups.

RESULTS

MTT results for anti-proliferative effects of single and combination agents

Both Cis and PAC-1 showed anti-proliferative effects in each of the cell lines tested. IC50 values of Cis in PC9, H1975, H1650, A549 and H1299 cells were 11.66, 7.62, 3.57, 4.92 and 1.89 μmol/L, and those of PAC-1 were 5.60, 14.80, 7.66, 7.36 and 9.68 μmol/L, respectively. Results are shown in Table 1 and Figure S1 (Supplementary material). For concurrent combination treatments, in each cell line, the IC25 or IC50 value of Cis...
in combination with 0.33, 1.0, 3.0, 9.0, 27.0 and 81.0 μmol/L PAC-1 were administered. For sequential combination treatments, in each cell line, parallel increasing doses of both drugs, that is, serial doses of Cis at 0.11, 0.33, 1.0, 3.0, 9.0 and 27.0 μmol/L in combination with 0.33, 1.0, 3.0, 9.0, 27.0 and 81.0 μmol/L PAC-1 were administered. For sequential combination treatments, three methods of treatment, 48 h of PAC-1 followed by 24 h of Cis, 24 h of Cis followed by 48 h of PAC-1 and 48 h of Cis plus PAC-1, were assessed. According to the differential sensitivities of each cell line to the drugs, the combination doses of PAC-1 and Cis were determined in these cell lines as described in the notes of Table 2 and Figure 1.

Additive effects were observed as we tested the fixed dose of IC_{50} or IC_{25} of cisplatin with variable doses of PAC-1 in H1299 cells. For concurrent combination treatments in H1299 cells, as shown in Table 2, with Cis IC_{25} or IC_{50} values plus the series of PAC-1 concentrations, most CI values were < 0.9, suggesting synergistic effects exerted by the concurrent combination of Cis plus PAC-1. When the doses of PAC-1 and Cis were increased in the same proportion in the concurrent combination treatments, the CI values increased to ~1.0, suggesting additive or even antagonistic effects at higher doses of the two drugs.

Notably, for the sequential combination treatments in H1299 cells, when sequential treatment of PAC-1 followed by Cis was used, most CI values were >1.1, indicating antagonistic anti-proliferative effects. However, when sequential treatment of Cis followed by PAC-1 was used, most CI values were <0.9, suggesting significant synergistic anti-proliferative effects. In other cell lines (PC9, H1975, H1650 and A549), the CI values of Cis and PAC-1 combination treatment, concurrent or sequential, were for the most part >1.1, indicating antagonistic effects with these combinations in EGFR or KRAS mutant cells. The results are shown in Tables S1, S2, S3 and S4.

### Flow cytometric analysis of cell cycle changes

Three independent flow cytometry experiments were conducted for each dosing condition to calculate a mean value. In Table S5, representative patterns of cell cycle data are shown. PAC-1 blocked cells at the S phase, whereas Cis inhibited the cell cycle at the G2 phase. In pan-negative H1299 cells (EGFR^{wt}, KRAS^{wt}, ALK^{wt}), sequential Cis→PAC-1 had the highest apoptotic rate of 20.7%.

### Detection of apoptosis-related proteins by Western blotting

As shown in Figure 2 and Figure S2, following the various treatments (Cis, PAC-1, Cis→PAC-1, PAC-1→Cis and Cis + PAC-1), proteins levels of CASP3, C-CASP3, XIAP, CASP9 and BCL-2 were measured in each cell line, with ACTB used as an internal control.

In H1299 cells (EGFR^{wt}, KRAS^{wt}, ALK^{wt}), treatment with PAC-1, or Cis in combination with PAC-1, concurrent or sequential, promoted CASP3 cleavage into the active form, C-CASP3 (CASP3 → C-CASP3 transformation), resulting in elevation of C-CASP3. In EGFR mutant PC9 or H1975 (L858R + T790M) cells, baseline CASP3 was converted to a certain degree into C-CASP3 when treated with PAC-1 alone or with PAC-1 in combination with Cis. The other molecules examined, BCL-2, XIAP and CASP9, were not affected significantly by the drug treatments.
Immunochemistry for apoptosis-related proteins

In each cell line, CASP3 and C-CASP3 were assessed by confocal microscopy. In H1299 cells, PAC-1 treatment significantly activated CASP3 into C-CASP3, in contrast to slight or no effect on C-CASP3 formation in the other cell lines (A549, H1650, H1975 and PC9 cells; Figure 3 and Figure S3).

DISCUSSION

Lung cancer is the leading cause of cancer-related deaths, both worldwide1,2 and in China.3 NSCLC is the major type, accounting for ~80% of lung cancers. Multidisciplinary comprehensive management is necessary for this malignancy. Chemotherapy remains the cornerstone for multiple-modality management of this malignancy at advanced stages, although in EGFR mutant or ALK-rearranged lung cancers, small-molecule inhibitors have been established as standards for care in targeted patient populations. For advanced lung cancer disease, other strategies are in the process of development, such as immuno-, anti-angiogenic, epigenetic and pro-apoptotic therapies.21,22

“Resisting cell death” is a hallmark of malignant cancer cells.10,11 There are basically two groups of regulatory and effector molecules: pro-apoptosis and anti-apoptosis molecules.23 Shifting the balance toward pro-apoptosis molecules is a fundamental strategy for overcoming the “resisting cell death” phenotype in cells, such as activating a pro-apoptotic process. Some studies have addressed this in lung cancer,24 but substantial evidence for the effectiveness of these approaches is lacking. CASP3 is the pivotal point in the apoptotic pathway, at the intersection of the external and internal apoptotic pathways, that relays the apoptotic signal to the nucleus. Activation of CASP3 may also sensitize cancer cells to other agents, such as chemotherapy.
Figure 1  Tumor cell growth inhibition by different groups of cisplatin in combination with PAC-1 in H1299 (a), PC9 (b), A549 (c), H1975 (d) and H1650 (e) cells. Constant ratio combination of Cis and PAC at molarity ratio 1:3 was used for tumor cell growth inhibition experiments for “Cis + PAC-1,” “Cis→PAC-1” and “PAC-1→Cis” groups. IC$_{25}$ Cis and IC$_{50}$ Cis were also used as fixed doses in combination with various concentrations of PAC-1 for non-constant ratio combination testing. The doses of Cis and PAC-1 were projected on the x-axis by log 10 transformation for constant intervals. (→) Cis+PAC-1, (←) Cis→PAC-1, (→) PAC-1→Cis, (←) IC$_{25}$ Cis+PAC1, (→) IC$_{50}$ Cis+PAC1.
In this study, we sought to analyze whether a caspase 3 activator, PAC-1, which activates procaspase-3 by chelating zinc and thus relieves the zinc-mediated inhibition of this zymogen, had anti-proliferative effects in lung adenocarcinoma cells, and whether there were synergistic effects in combination with Cis, a key chemotherapeutic agent, in these cancer cells. Our IHC data regarding CASP3 expression showed that CASP3 was
overexpressed in some lung cancers (Fig. S4). Activation of CASP3 in these cancers might have the potential for improving treatment outcomes. Here, we used cell lines with different genetic alterations: H1299 (EGFRwtKRASmutALKwt), A549 (KRASmut), PC9 (EGFRmut), H1650 (EGFRmut) and H1975 (EGFRmut). Single-agent PAC-1 or Cis had anticancer effects in all cell lines with moderately different IC50 values, with that the IC50 values in these cell lines were 1.9–11.7 μmol/L for Cis and 5.6–14.8 μmol/L for PAC-1. For combination effects, we tested both concurrent and sequential combinations of PAC-1 with Cis. For sequential treatments, three methods were used as reported previously.18 In other reports, several sequential combination procedures had been used. Examples include addition of the first drug for 24 h followed by the second drug for a further 48 h,29 treatment of the first drug for 24 h followed by its removal and addition of the second drug for a further 48 h,27 targeted therapy for 72 h followed by chemo treatment for 24 h or chemo treatment for 24 h followed by targeted therapy for 48 h,28 and the first drug for 24 h followed by a second single or add-on drug for 24 h.29,30

Different means of administration of the drugs may affect the anti-proliferative outcome. Here we used PAC-1 treatment for 48 h and Cis treatment for 24 h (72 h total). Under this regimen, notably, only in H1299 cells (without EGFR, KRAS or ALK gene alterations) did we see any synergistic effects of PAC-1 with Cis; when administered sequentially (Cis followed by PAC-1), the CI value was <0.9.

With the concurrent combination treatments in H1299 cells, whether at the Cis IC25 or IC50 values plus various concentrations of PAC-1, most CI values were <0.9, suggesting synergistic effects. However, when we increased the doses of both drugs, antagonistic effects occurred. In the other cell lines (PC9, H1975, H1650 and A549), the CI values of Cis in combination with PAC-1, whether concurrent or sequential combinations, were for the most part >1.1, indicating antagonistic effects in these EGFR or KRAS mutant cells though Cis though sequential Cis→PAC-1 or concurrent Cis + PAC-1 showed a trend of enhanced inhibition in PC9 cells.

To examine the mechanism of action of PAC-1 and/or Cis treatment, flow cytometry was used to assess changes in cell cycle status. Western blot and immunochemical assays for CASP3 and other molecules were also conducted. In all five cell lines, PAC-1 inhibited the cell cycle at the S phase, while Cis inhibited the cell cycle at the G2 phase. In theory, Cis would have caused DNA adducts or cross-links and damage, leading to a stop in the cell cycle at the G2/M phase. PAC-1 acted on the apoptotic pathway to promote CASP3 activity, which could lower the threshold for apoptosis in the cells, leading to a blockade in the S phase. When targeted therapies are combined with cytotoxic chemotherapy, due to the actions at different cell cycle checkpoints, the pharmacodynamic separation phenomenon can possibly occur.31–33 That is, some combinations may not be synergistic as expected. For example, EGFR-TKI in concurrent combination with chemotherapy in unselected patients showed no benefit.34–36 Certain sequential regimens might be effective because of differential actions at different cell cycle phases. As shown previously,30 chemotherapeutic reagents act mainly on G2/M blockade and increase the activity of receptor kinases, such that after chemotherapy, cells may be inhibited by EGFR TKI at the G1 phase.37,38 Based on these results, sequential chemotherapy with EGFR TKI was proposed for the FASTACT studies and was confirmed to be beneficial in the clinic.39 In our study, PAC-1 was used as a targeted therapy, and it blocked cell cycle similarly to the EGFR TKIs but mainly at S phase with unknown mechanisms. Thus, concurrent Cis plus PAC-1 treatment had synergistic effects but became antagonistic with increased combination doses, and sequential Cis to PAC-1 was the best combination regimen evaluated, causing a high apoptotic rate in cancer cells. To further explore the synergistic action of PAC-1+Cis at a molecular level in H1299 cells, Western blot analysis was used to assess protein expression patterns of CASP3, CASP9, C-CASP3, XIAP and BCL2. In H1299 cells, PAC-1 alone or in concurrent or sequential combination with Cis could induce C-CASP3 levels, consistent with a previous report.40 Mechanistically, PAC-1 may bind Zn ions competitively and promote cleavage of CASP3 into C-CASP.41

PAC-1-mediated C-CASP3 activation was also confirmed by confocal microscopy in our study. In EGFRwtKRASmutALKwt H1299 cells, PAC-1 induced greater levels of C-CASP3 compared with EGFR or KRAS mutant cells, suggesting that in H1299 cells, CASP3 might be activated more readily by PAC-1, consistent with the flow cytometry results, which showed sequential treatment of Cis followed by PAC-1 could cause a high rate of apoptosis in H1299 cells.

Limitations of this study included a lack of testing in other wild-type cells, data from animal models and data clarifying the mechanisms of the synergistic action of PAC-1 with Cis in wild-type cells.
Synergistic activity of PAC-1 and cisplatin

In summary, our results demonstrated that CASP3 might be an actionable target in some pulmonary adenocarcinomas such as H1299 cells with EGFR\textsuperscript{wt}/KRAS\textsuperscript{wt}ALK\textsuperscript{wt}. Cis in combination with PAC-1 could have better synergistic anti-cancer effects in H1299 cells compared with other KRAS or EGFR mutant cell lines. In EGFR mutant H1650, and H1975 cells and in KRAS mutant A549 cells, Cis in combination with PAC-1 showed antagonistic effects in terms of anti-proliferation. PAC-1 could induce activation of C-CASP3 and block tumor cells in the S phase, and it might have a specific effects profile when combined with Cis.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: J-QH and H-LL. Performed the experiments: H-LL, ZX, and T-EJ. Analyzed the data: H-LL, X-CZ, ZX and J-QH. Contributed reagents/materials/analysis tools: H-LL, T-EJ. Analyzed the data: H-LL, X-CZ, ZX and J-QH. Performed the experiments: H-LL, ZX, and J-QH. Wrote the paper: H-LL, X-CZ, and J-QH.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site: Figure S1 Tumor cell growth inhibition by serial single-drug concentrations of cisplatin or PAC-1 in H1299 (a), PC9 (b), A549 (c), H1975 (d) and H1650 (e) cells. The doses of Cis and PAC-1 were projected on the X-axis by log 10 transformation for constant intervals.

Figure S2 Immunoblotting results of apoptotic molecules CASP3, C-CASP3, XIAP, CASP9 and BCL2 in PC9 cells treated by PAC-1 at different time points (c) and treated by cisplatin, PAC-1 and cisplatin in combination with PAC-1 (b). Immunoblotting results of apoptotic molecules CASP3, C-CASP3, XIAP, CASP9 and BCL2 in H1975 cells treated by PAC-1 at different time points (c) and treated by cisplatin, PAC-1 and cisplatin in combination with PAC-1 (d). The cells were treated with the IC50 value of Cis or PAC-1 or combinotorial Cis with PAC-1 for a total of 72 h.

Figure S3 Immunofluorescent cytochemistry results of CASP3 and C-CASP3 in control (a, b) and PAC-1-treated (c, d) A549 cells. Immunofluorescent cytochemistry results of CASP3 and C-CASP3 in control (e, f) and PAC-1-treated (g, h) PC9 cells. Immunofluorescent cytochemistry results of CASP3 and C-CASP3 in control (i, j) and PAC-1-treated (k, l) H1650 cells.
cent cytochemistry results of CASP3 and C-CASP3 in control (m, n) and PAC-1-treated (o, p) H1975 cells. Figure S4 Representative photographs of CASP3 staining by IHC as – (a), + (b), ++ (c), +++ (d) in tissue microarray. Over-expression was defined as more than 20% of tumor cells with staining intensity of greater than 2 plus (++) and expression rate was 36.8% (53/144).

Table S1 Combination index (CI) of different groups of cisplatin in combination with PAC-1 in PC9 cells (mean ± SE).

Table S2 Combination index (CI) of different groups of cisplatin in combination with PAC-1 in A549 cells (mean ± SE).

Table S3 Combination index (CI) of different groups of cisplatin in combination with PAC-1 in H1650 cells (mean ± SE).

Table S4 Combination index (CI) of different groups of cisplatin in combination with PAC-1 in H1975 (mean ± SE).

Table S5 Cell cycle analysis in control cells and those treated with Cis, PAC-1, Cis→PAC-1, PAC-1→Cis and PAC-1 + Cis (mean ± SD, n = 3).