In vitro synergistic antitumor efficacy of sequentially combined chemotherapy/icotinib in non-small cell lung cancer cell lines

MIN-CONG WANG¹, XUAN LIANG¹, ZHI-YAN LIU¹,², JIE CUÍ¹,², YING LIU¹, LI JING¹, LI-LI JIANG¹, JIE-QUN MA¹, LI-LI HAN¹, QIAN-QIAN GUO¹, CHENG-CHENG YANG¹, JING WANG¹, TAO WU¹, KE-JUN NAN¹ and YU YAO¹

¹Department of Oncology, The First Affiliated Hospital, College of Medicine of Xi’an Jiaotong University; ²Department of Oncology, Zhong Xin Hospital, Xi’an; ³Department of Oncology, Yanan University Affiliated Hospital, Yan’an, Shaanxi, P.R. China

Received May 28, 2014; Accepted August 25, 2014

DOI: 10.3892/or.2014.3583

Abstract. The concurrent administration of chemotherapy and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) has previously produced a negative interaction and failed to confer a survival benefit to non-small cell lung cancer (NSCLC) patients compared with first-line cytotoxic chemotherapy. The present study aimed to investigate the optimal schedule of the combined treatment of cisplatin/paclitaxel and icotinib in NSCLC cell lines and clarify the underlying mechanisms. HCC827, H1975, H1299 and A549 human NSCLC cell lines with wild-type and mutant EGFR genes were used as in vitro models to define the differential effects of various schedules of cisplatin/paclitaxel with icotinib treatments on cell growth, proliferation, cell cycle distribution, apoptosis, and EGFR signaling pathway. Sequence-dependent antiproliferative effects differed among the four NSCLC cell lines, and were not associated with EGFR mutation, constitutive expression levels of EGFR or downstream signaling molecules. The antiproliferative effect of cisplatin plus paclitaxel followed by icotinib was superior to that of cisplatin or paclitaxel followed by icotinib in the HCC827, H1975, H1299 and A549 cell lines, and induced more cell apoptosis and G0/G1 phase arrest. Cisplatin and paclitaxel significantly increased the expression of EGFR phosphorylation in the HCC827 cell line. However, only paclitaxel increased the expression of EGFR phosphorylation in the H1975 cell line. Cisplatin/paclitaxel followed by icotinib influenced the expression of p-EGFR and p-AKT, although the expression of p-ERK1/2 remained unchanged. The results suggest that the optimal schedule of the combined treatment of cisplatin/paclitaxel and icotinib differed among the NSCLC cell lines. The results also provide molecular evidence to support clinical treatment strategies for NSCLC patients.

Introduction

Lung cancer is one of the leading causes of cancer mortality in developed countries, and non-small cell lung cancer (NSCLC) accounts for 80-85% of lung cancer cases (1). The majority of NSCLC patients have locally advanced or distant metastatic disease at the time of presentation and thus cannot undergo surgery (2). Platinum-based doublet chemotherapy is the mainstay of treatment for advanced NSCLC (3). However, it has significant side-effects and a 5-year survival rate of only 20% (4). Previous findings suggest that the chemotherapeutic treatments of NSCLC have reached a therapeutic plateau (5,6). Thus, exploring new effective integrated treatment methods to improve the tumor response rate and prolong the survival time of advanced NSCLC patients is important.

The epidermal growth factor receptor (EGFR) pathway has been shown to be an important target in NSCLC proliferation. The emergence of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) offers new hope to patients with advanced NSCLC patients. In 2004, it was reported that tumors with EGFR-activating mutations had histological characteristics of adenocarcinoma, and were highly sensitive to EGFR-TKIs with a better prognosis as compared to the EGFR wild-type (7-9). The efficiency of EGFR-TKIs reached 70-80% and the median survival time was 20-30 months (10,11). EGFR-TKIs are superior to cisplatin plus paclitaxel as an initial treatment for patients with advanced NSCLC harboring an EGFR mutation (12). Two randomized studies (WJTOG3405 and NEJ) showed that patients with an EGFR mutation have a high tumor response rate and progression-free survival (PFS) than those with EGFR-TKIs (13,14). EGFR-TKIs provide a new option to patients since they can prolong survival and significantly improve the quality of life (15).
The combination of EGFR-TKIs with chemotherapy is not more beneficial than chemotherapy alone (INTACT-1 and INTACT-2, TRIBUTE and TALENT) (16-19). An antagonistic effect exists between EGFR-TKIs and chemotherapy drugs. The failure to achieve positive results may be due to not being able to select EGFR-sensitizing mutations and using inappropriate drug administration sequences, thereby leading to cell cycle-specific antagonism (20-24).

Icotinib, an oral EGFR-TKI, has shown antitumor activity and favorable toxicity in early phase clinical trials. To assess the safety and tolerability of icotinib, Zhao et al selected NSCLC patients after the failure of prior platinum-based chemotherapy (25). Their results showed that oral icotinib is generally well tolerated with manageable and reversible adverse events, and shows positive clinical antitumor activities in patients with advanced NSCLC (25). A randomized, double-blind phase 3 non-inferiority trial showed that icotinib is non-inferior to gefitinib in terms of PFS, suggesting that icotinib is a new treatment option for pretreated patients with advanced NSCLC (26).

In the present study, we used human EGFR wild-type and mutant NSCLC cell lines to define the differential effects of cisplatin, paclitaxel and icotinib in different schedules on cell growth proliferation, cell cycle distribution, apoptosis and signaling pathways. Specifically, we tested the effects of cisplatin plus paclitaxel combined with icotinib in different schedules.

Materials and methods

Drugs. Icotinib, kindly provided by the Beida Pharmaceutical Company (China), was dissolved in 20 mM dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) as stock solution. Cisplatin and paclitaxel were purchased from Sigma, and respectively dissolved in 1 mM DMSO as stock solution. The drugs were diluted with culture medium before use.

Cell lines. HCC827, H1975, H1299 and A549 human NSCLC cell lines were obtained from the Chinese Academy of Sciences Institute of Life Sciences Cell Resource Center in Shanghai. The cell lines were grown in RPMI-1640 medium (Sciences Institute of Life Sciences Cell Resource Center in cell lines were obtained from the Chinese Academy of Sciences Institute of Life Sciences Cell Resource Center in Shanghai). Their results showed that oral icotinib is generally well tolerated with manageable and reversible adverse events, and shows positive clinical antitumor activities in patients with advanced NSCLC (25). A randomized, double-blind phase 3 non-inferiority trial showed that icotinib is non-inferior to gefitinib in terms of PFS, suggesting that icotinib is a new treatment option for pretreated patients with advanced NSCLC (26).

Materials and methods

Drugs. Icotinib, kindly provided by the Beida Pharmaceutical Company (China), was dissolved in 20 mM dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) as stock solution. Cisplatin and paclitaxel were purchased from Sigma, and respectively dissolved in 1 mM DMSO as stock solution. The drugs were diluted with culture medium before use.

Cell lines. HCC827, H1975, H1299 and A549 human NSCLC cell lines were obtained from the Chinese Academy of Sciences Institute of Life Sciences Cell Resource Center in Shanghai. The cell lines were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 U/ml penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO₂. Cells in the exponential growth phase were harvested using trypsin-EDTA.

Gene sequencing. Genomic DNA was extracted from the HCC827, H1975, H1299 and A549 cell lines using the ABI Prism™ Primer Express software (Applied Biosystems, Foster City, CA, USA) and amplified polymerase chain reaction of genomic DNA. The samples of positive amplified bands were then sequenced.

Evaluation of antiproliferative effects. Cell viability was determined using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded at ~5,000/well in 96-well plates. At 24 h after seeding, the cells were exposed to the drugs. To evaluate the single-agent treatment, the cells were exposed to icotinib, cisplatin or paclitaxel alone for 72 h, and the half maximal inhibitory concentration (IC₅₀) was considered as the concentration resulting in 50% cell growth inhibition compared with the untreated control cells. To evaluate the antiproliferative effects of the combined treatment, the cells were treated with three different sequences: i) pretreated with cisplatin/paclitaxel for 24 h and washed once with phosphate-buffered saline (PBS), followed by icotinib for 48 h; ii) pretreated with icotinib for 48 h and washed once, followed by cisplatin/paclitaxel for 24 h; iii) treated concomitantly with cisplatin/paclitaxel and icotinib for 48 h, and incubated in drug-free medium for 24 h. At 72 h after drug treatment, the cells were washed once with PBS and incubated with medium containing MTT (0.5 mg/ml in medium) for 4 h at 37°C. The culture medium with MTT was removed, and formazan crystals were reabsorbed in 200 µl of DMSO (Sigma). Cell viability was determined by measuring the absorbance at 570 nm. Experiments were conducted on at least three separate occasions. Thus, we used 0.125-, 0.25-, 0.5-, 1-, 2- and 4-fold the IC₅₀ dose in cisplatin/paclitaxel and icotinib combination doses were used to calculate the combination index (CI) value. The CI was calculated using CompuSyn software (ComboSyn, Inc., Paramus, NJ, USA). The resulting CI was a quantitative measure of the degree of interaction between different drugs, with CI >1.0, CI=1.0 and CI <1.0, indicating antagonistic, additive and synergistic effects, respectively.

Clonogenic survival assays. To investigate the effects of chemotherapy followed by icotinib on the NSCLC cell lines, a standard clonogenic assay was performed. The cells were seeded in triplicate in 6-well plates (5x10⁴ cells/well) and treated with DMSO as the vehicle control. After exposure to cisplatin/paclitaxel at IC₅₀ levels for 24 h, the cells were washed and exposed to icotinib at IC₅₀ levels for 48 h. The cells were then washed and incubated in drug-free medium for 14 days. Colonies were stained with crystal violet and manually counted. All of ≥50 cells were counted. The survival fraction (SF) was estimated based on the formula: SF = number of colonies formed/number of cells seeded x plating efficiency of the control group. All the experiments were performed in triplicate.

Analysis of cell cycle and apoptosis. Cells were seeded in 6-well plates at a density of 1x10⁵/well. After 24 h, the cells were treated with cisplatin/paclitaxel and icotinib sequentially at IC₅₀ levels. To analyze the cell cycle, cells were trypsinized, washed two times with PBS and harvested by centrifugation after the treatments were completed. The cells were then fixed with 70% ice-cold ethanol for at least 1 h, centrifuged, washed two times in cold PBS, stained with propidium iodide (PI) solution (0.05 mg/ml PI and 10 mg/ml RNase A) for 20 min at 37°C in the dark, and analyzed using a flow cytometer (FACSCalibur; Becton-Dickinson Biosciences, San Jose, CA, USA). To analyze cell apoptosis, adherent and non-adherent cells were harvested after the drug treatments, washed with cold PBS, stained with Annexin V-fluorescein isothiocyanate (FITC) and PI (Joincare Medicine Company, China) for 15 min at 37°C in the dark and analyzed using a flow cytometer (FACSCalibur).
Table I. IC<sub>50</sub> values for each drug were calculated by performing dose-response experiments with cisplatin, paclitaxel and icotinib.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Cisplatin</th>
<th>Paclitaxel</th>
<th>Icotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC827</td>
<td>4.9 µmol/l</td>
<td>1.6 nmol/l</td>
<td>290 nmol/l</td>
</tr>
<tr>
<td>H1975</td>
<td>11.0 µmol/l</td>
<td>1.7 nmol/l</td>
<td>8.8 µmol/l</td>
</tr>
<tr>
<td>H1299</td>
<td>7.8 µmol/l</td>
<td>13.3 nmol/l</td>
<td>25.9 µmol/l</td>
</tr>
<tr>
<td>A549</td>
<td>5.7 µmol/l</td>
<td>2.3 nmol/l</td>
<td>6.9 µmol/l</td>
</tr>
</tbody>
</table>

Western blot analysis. After the drug treatments, the cells were harvested in ice-cold PBS and lysed with RIPA cell lysis buffer containing a protease inhibitor cocktail. The protein concentration was determined using the BCA protein assay reagent (both from Wolsen Company, China). Each protein sample was resolved on sodium dodecyl sulfate polyacrylamide gels (8%), transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Billerica, MA, USA), blocked for 1 h at room temperature in 5% non-fat milk, and incubated with the appropriate primary antibodies according to the manufacturer's instructions. The primary antibodies EGFR, p-EGFR, AKT, p-AKT, ERK1/2, p-ERK1/2 and β-actin were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The blots were then washed with TBST for 30–40 min, and incubated with the secondary antibody (Wolsen Company) at room temperature for 1 h. Secondary antibodies were also purchased from Cell Signaling Technology, Inc. For the quantification of protein levels, films were scanned and analyzed using Labworks software.

Statistical analysis. Data are expressed as the means ± standard deviation (SD) of at least three experiments. Statistically significant differences between groups were determined by the Student’s t-test using SPSS 19.0 software. In each case, p<0.05 was considered to indicate a statistically significant result.

Results

EGFR gene sequencing. The specific primers used were for amplifying the cDNA fragments of the EGFR tyrosine kinase domain. Fig. 1 shows that the HCC827 cell line harbors the exon 19 sequence deletion. The H1975 cell line harbors the exon 20 sequence T790M and exon 21 sequence L858R mutation. The H1299 and A549 cell lines were wild-types of EGFR.

Drug sensitivities of the HCC827, H1975, H1299 and A549 cell lines. Treatment with cisplatin, paclitaxel or icotinib alone for 72 h resulted in the dose-dependent inhibition of NSCLC cell growth. Table I summarizes the IC<sub>50</sub> values of the three drugs. The four cell lines showed sensitivities similar to cisplatin and paclitaxel. The HCC827 cell line was highly sensitive to icotinib, whereas the H1975, H1299 and A549 cell lines showed resistance to icotinib.

Constitutive expression levels of EGFR and downstream signaling molecules in four NSCLC cell lines. We compared the basal EGFR expression levels of the NSCLC cell lines by western blotting. As shown in Fig. 2, the HCC827 cell line had significantly higher levels of EGFR and p-EGFR than A549, H1975 and H1299 cell lines. We also observed constitutive AKT and ERK1/2 phosphorylation in the four NSCLC cell lines. The basal AKT and p-AKT levels were similar in the four cell lines, although these levels were slightly lower in the HCC827 cell line. The ERK1/2 and p-ERK1/2 levels in the HCC827 cell line were higher than those in the other cell lines.

Sequence of cisplatin/paclitaxel followed by icotinib is more effective than other sequences in the NSCLC cell lines. To evaluate the antiproliferative effects of cisplatin, paclitaxel and icotinib treatments, we performed a series of MTT cell growth assays. We evaluated the antiproliferative effects on the HCC827, H1975, H1299 and A549 cell lines in three different sequences. As shown in Fig. 3A, the antiproliferative effects between cisplatin/paclitaxel and icotinib were sequence-dependent. Although the differences were not significant, the sequence of cisplatin/paclitaxel followed by icotinib was better than other sequences in the HCC827, H1975, H1299 and A549 cell lines.

The combined effect of cisplatin/paclitaxel and icotinib was evaluated on the basis of the CI (Fig. 4). In the HCC827 cell line, which was highly sensitive to EGFR-TKIs, the sequence of cisplatin followed by icotinib resulted in a synergistic antiproliferative effect (CI <1). By contrast, the sequence of icotinib followed by cisplatin resulted in an antagonistic interaction (CI >1). However, three different sequences of combined paclitaxel and icotinib resulted in an antagonistic and synergistic interaction with the increasing drug concentration. The IC<sub>50</sub> values of cisplatin or paclitaxel followed by icotinib and concomitant administration resulted in a synergistic effect (CI <1), whereas the sequence of icotinib followed by cisplatin resulted in an antagonistic interaction (CI >1). Three different sequences of combined paclitaxel and icotinib resulted in antagonistic and synergistic interactions with the increasing drug concentration. The H1299 and A549 cell lines were wild-types of EGFR. In the H1299 cell line, a synergistic antiproliferative effect was observed with the sequence of cisplatin followed by icotinib and concomitant administration (CI <1). The sequence of icotinib followed by cisplatin resulted in an antagonistic interaction (CI >1). However, all three different sequences of combined paclitaxel and icotinib resulted in an antagonistic interaction. In the A549 cell line, the three different sequences of combined cisplatin/paclitaxel and icotinib resulted in synergistic and antagonistic interactions with the increasing drug concentration.

Three-drug combination is better than two-drug combination in the HCC827, H1975 and A549 cell lines, but not in the H1297 cell line. The antiproliferative effects of cisplatin plus paclitaxel followed by icotinib were compared with those of cisplatin or paclitaxel followed by icotinib. We determined that the antiproliferative effects of cisplatin plus paclitaxel followed by icotinib were better than those of cisplatin or paclitaxel followed by icotinib in the HCC827, H1299 and A549 cell lines (Fig. 5A). We also evaluated the effects of
cisplatin plus paclitaxel followed by icotinib in the HCC827 and H1975 cell lines using a clonogenic assay (Fig. 5B and C). Cells were exposed to cisplatin, paclitaxel and icotinib at the \( IC_{50} \) values. The combinations, regardless of whether they were three-drug or two-drug, decreased the survival rates of HCC827 and H1975 cells compared with the control group. Clonogenic survival of cisplatin plus paclitaxel followed by icotinib was the lowest compared with that of cisplatin or paclitaxel followed by icotinib in the HCC827 cell line (three-drug combination vs. two-drug combination, \( p<0.05 \)), but not in the H1975 cell line (\( p>0.05 \)).

Three-drug combination induced more G0/G1 phase arrest and apoptosis than the two-drug combination in the HCC827 cell line, but not in the H1975 cell line. DNA flow cytometry studies were performed to evaluate the effect of drug
Figure 3. Antiproliferative effects of cisplatin/paclitaxel combined with icotinib are sequence-dependent. (A) Schema of sequential treatment. (B) Sequence of cisplatin/paclitaxel followed by icotinib produced the most potent antiproliferative effect in the NSCLC cell lines. P/T-I, cisplatin/paclitaxel followed by icotinib; P/T+I, concomitant cisplatin/paclitaxel and icotinib; and I-P/T, icotinib followed by cisplatin/paclitaxel. Data are presented as the means ± SD from three independent experiments. NSCLC, non-small cell lung cancer.
combinations on the cell cycle distribution. We selected the HCC827 and H1975 cell lines to determine whether their cell cycle-modulating activity provides evidence clues to optimize drug scheduling. The cells were exposed to cisplatin, paclitaxel and icotinib at the IC_{50} values. All the agents affected the cell cycle of the HCC827 and H1975 cell lines (Fig. 6A).
In response to the treatment of cisplatin followed by icotinib, cell fractions in the S phase decreased (26±2.0 and 15±1.8%), whereas those in the G0/G1 phase increased (63±1.4 and 69±1.8%) compared with the control group in the HCC827 and H1975 cell lines, respectively. In response to the treatment of paclitaxel followed by icotinib, cell fractions in the S phase also decreased (21±1.5 and 14±1.3%), whereas those in the G0/G1 phase increased (64±1.9 and 71±2.0%). After the treatment with cisplatin plus paclitaxel followed by icotinib, the proportion of the HCC827 cell line in the G0/G1 phase significantly increased compared with that in the other treatment groups (72±0.8%, p<0.05). However, no difference was observed in the H1975 cell line (71±1.7%, p>0.05).

Effect of cisplatin, paclitaxel and icotinib on EGFR and downstream signaling molecules in the HCC827 and H1975 cell lines.
To gain insight into the mechanisms involved in regulating the interaction of cisplatin, paclitaxel and icotinib, we examined the effects on EGFR and downstream signaling molecules in the HCC827 and H1975 cell lines (Fig. 7). The cells were exposed to the IC$_{50}$ doses of drugs. Exposure to cisplatin, paclitaxel or icotinib alone resulted in no changes in the total proteins of EGFR, AKT and ERK1/2 in the HCC827 and H1975 cell lines. The HCC827 cell line exhibited increases in p-EGFR and p-AKT in response to cisplatin alone, although p-ERK1/2 was unchanged. When the HCC827 cell line was exposed to paclitaxel alone, the p-EGFR levels significantly increased, whereas the p-AKT and p-ERK1/2 levels were unchanged. We observed that icotinib significantly inhibited p-EGFR, p-AKT and p-ERK1/2 in the HCC827 cell line (Fig. 7A). In the H1975 cell line, we observed an increase in the p-AKT level after cisplatin. In addition, the paclitaxel-treated H1975 cell line showed an increase in the p-EGFR and p-AKT levels. Icotinib-treated H1975 cells showed a decrease in the p-EGFR and p-AKT levels, although the p-ERK1/2 level was unchanged (Fig. 7B).

We also examined the effect of cisplatin/paclitaxel followed by icotinib on EGFR and downstream signaling molecules in the HCC827 and H1975 cell lines (Fig. 7C). In the HCC827 and H1975 cell lines, the combinations of cisplatin/paclitaxel followed by icotinib affected the expression of p-EGFR and p-AKT, but not p-ERK1/2. In the HCC827 cell line, the expression levels of p-EGFR and p-AKT with cisplatin plus paclitaxel followed by icotinib were significantly lower than those of cisplatin or paclitaxel followed by icotinib (p<0.05), although not in the H1975 cell line.

**Discussion**

The present study aimed to investigate the optimal schedule of combined treatment with cisplatin/paclitaxel and icotinib in NSCLC cell lines, and gain insight into the molecular mechanisms underlying the interaction of these drugs *in vitro*. In the present study, we observed that the sequence of cisplatin followed by icotinib resulted in a synergistic effect on the HCC827, H1975 and H1299 cell lines. In addition, paclitaxel followed by icotinib showed a synergistic effect in the HCC827 and H1975 cell lines at high concentrations. However, the sequences of cisplatin followed by icotinib and paclitaxel followed by icotinib resulted in synergistic effects only in low concentrations in the A549 cell line. The antiproliferative effect of cisplatin plus paclitaxel followed by icotinib was superior to that of cisplatin or paclitaxel followed by icotinib in the HCC827, H1299 and A549 cell lines, although not in the H1975 cell line harboring the T790M and L858R mutations. This antiproliferative effect seemed to have no correlation with the constitutive expression levels of EGFR and downstream signaling molecules in the four NSCLC cell lines. We also determined that the potentiation of the antiproliferative activity of EGFR-TKIs and chemotherapy in combination was sequence-dependent.

In previous studies sequence-dependent interactions between EGFR-TKIs and chemotherapy in human cancer cell lines were shown (27). Cheng *et al* observed that the sequence of paclitaxel followed by gefitinib is an appropriate treatment combination that is superior to other sequences in treating...
Figure 7. Effects of cisplatin, paclitaxel and icotinib alone or in combination on EGFR and downstream signaling molecular expression in the HCC827 and H1975 cell lines. (A) The HCC827 cell line was treated with cisplatin, paclitaxel and icotinib alone. (B) The H1975 cell line was treated with cisplatin, paclitaxel and icotinib alone. (C) The effect of cisplatin/paclitaxel followed by icotinib on EGFR and downstream signaling molecular expression in the HCC827 and H1975 cell lines. The HCC827 and H1975 cell lines were treated with IC_{50} value of drugs. The blots are from one typical experiment. Data are presented as the means ± SD from three independent experiments. Statistically significant differences are presented as *p<0.05. EGFR, epidermal growth factor receptor.
the NSCLC cell lines (28,29). Tsai et al determined that the concomitant gefitinib/cisplatin combination shows antagonism in the majority of sensitizing mutations of EGFR wild-type or NSCLC cells, and the three-drug combination is not better than the two-drug combination (30). The present study was innovative since we determined that cisplatin plus paclitaxel followed by icotinib was superior to cisplatin or paclitaxel followed by icotinib in some of the NSCLC cell lines. In addition, the EGFR signaling pathway may have a function in the sequence-dependent interaction in the NSCLC cell lines.

Given that the INTACT-1, INTACT-2, TALENT and TRIBUTE clinical trials were unsuccessful, Gandara et al (21,31) suggested two hypotheses that likely explain these negative results. Firstly, patients were not selected based on a predictive response marker. Secondly, the potentiation of negative results. Firstly, patients were not selected based on (21,31) suggested two hypotheses that likely explain these negative results. Firstly, patients were not selected based on.

In conclusion, the present study has demonstrated that the most advantageous schedule to treat NSCLC in vitro was the sequence of cisplatin/paclitaxel followed by icotinib. We also characterized the molecular mechanisms involved in the synergistic effect between cisplatin/paclitaxel and icotinib against the NSCLC cell lines. Although the extrapolation of in vitro data to the clinical setting should be considered with caution, these results may provide a rationale for the ongoing clinical investigation of the sequential treatment of NSCLC.

Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (no. 81101777), and the social development research project of Shaanxi Provincial Department of Science and Technology (no. 2013K12-08-3).

References


