The Human Lung Cancer Drug Resistance-Related Gene BC006151 Regulates Chemosensitivity in H446/\textsubscript{c}DDP Cells

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This study aimed to investigate the mechanism by which the human lung cancer drug resistance-related gene BC006151 regulates chemosensitivity by down-regulating BC006151 expression via antisense gene transfer in H446/\textsubscript{c}DDP cells. A retroviral vector containing the antisense BC006151 sequence was constructed and transferred into H446/\textsubscript{c}DDP cells. Transfection of the empty vector served as a negative control. The two groups of transfected cells were treated with various chemotherapeutic agents, after which morphological changes in cell ultrastructure were compared by transmission electron microscopy, cell proliferation and chemosensitivity to particular chemotherapeutic agents were compared by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method, the effects of chemotherapy on cell cycle and apoptosis were compared by flow cytometry, and Bel-2 was evaluated by immunohistochemistry and Western blot analysis. Results showed that apoptotic body-like structures were identified by transmission electron microscopy in the antisense gene-transfected cells. MTT founded that these cells exhibited a significantly lower level of proliferation than the control cells (p < 0.01), together with a markedly increased sensitivity to various chemotherapeutic agents (p < 0.01). Flow cytometry analysis revealed that a G1 phase arrest accounted for the reduction in proliferation in the antisense gene-transfected cells; increased apoptosis was also detected (p < 0.01). Both immunohistochemistry and western blot analysis confirmed that Bel-2 expression was significantly down-regulated in the antisense gene-transfected cells compared to controls. In a word, down-regulation of BC006151 can significantly inhibit proliferation and increase apoptosis of H446/\textsubscript{c}DDP cells after chemotherapy, and this gene may play an important role in the development of multidrug resistance in lung cancer.

Key words BC006151 gene; lung cancer; multidrug resistance; proliferation; apoptosis

Lung cancer is a highly malignant neoplasm. In China, the mortality rate associated with lung cancer increased from 7.17 per 10 million in the 1970s to 15.2 per 10 million in the 1990s, equivalent to a 111.9% increase. It is also the leading cause of cancer death in the urban population.\textsuperscript{1,2} Moreover, 75% of patients have already progressed to the late stage of the disease by the time they are admitted, and have thus lost the opportunity for surgical intervention. Therefore, chemotherapy has become an important therapeutic approach to lung cancer, as it can significantly increase the general survival rate of lung cancer patients as well as alleviate their symptoms and improve their quality of life.\textsuperscript{3,4} However, one of the major reasons for chemotherapy failure in lung cancer is the drug resistance of cancer cells. Many previous studies have found that the development of drug resistance in lung cancer is closely related to the dysregulated expression of multidrug resistance (MDR) proteins, P-glycoprotein (P-gp), topoisomerase (Topo II), glutathione (GSH), and glutathione GSH-S-transferase (GST).\textsuperscript{5–8} Studies using molecular biological techniques and antisense gene transfer to block the expression of lung cancer drug resistance-related genes may help to explicate the mechanisms by which these genes regulate cancer cell proliferation and apoptosis, and they may provide a new theoretical basis for the clinical reversal of MDR in lung cancer.

In previous studies, our group cloned the full length cDNA of the BC006151 gene by suppression subtractive hybridization (SSH) and confirmed that its expression was significantly up-regulated during \textsubscript{c}DDP-induced drug resistance in a human lung adenocarcinoma cell line, SPC-A-1 and a small cell lung cancer cell line, H446.\textsuperscript{9,10} These results prompted us to identify BC006151 as a new lung cancer drug resistance-related gene. In the present study, we used a retroviral vector to transfect the BC006151 antisense sequence into the drug-resistant small cell lung cancer cell line, H446/\textsubscript{c}DDP, to study the effects of BC006151 inhibition on the chemosensitivity of these cells. We sought to establish an empirical basis for further investigations of the function of this novel lung cancer drug resistance gene and to open new avenues for exploring drug resistance mechanisms in lung cancer cells.

MATERIALS AND METHODS

Tissue Collection Six fresh, paired samples of lung cancer tissue and adjacent non-cancerous tissue were collected from patients of the Department of Thoracic Surgery at Xinqiao Hospital in the Third Military Medical University. The mean age was 58 years (range, 40—72 years) with 2 women and 4 men. All tissues were stored at −70 °C, and all cancers samples were clinicopathologically confirmed to be small cell lung cancer (SCLC). The 6 patients had not been treated by radiotherapy and chemotherapy before operation. All protocols were approved by the hospital’s Protection of

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Human Subjects Committee. The use of human tissues was approved by the institutional review board of the Third Military Medical University, and all tissue samples were processed following international guidelines.

**Construction of the BC006151 Antisense Expression Vector, pCMV-Script and Quantitative Analysis of BC006151 mRNA** The primer sequences used to amplify BC006151 mRNA were 5'-GAAAGCTTAAAGACAGAG-GAGGCT-3' and 5'-CCGGATCCAGATGCTTTTAGTT-3'. A HindIII restriction site was engineered in the 5' primer, while a BamHI site was engineered in the 3' primer. The BC006151 sequence was amplified by conventional reverse transcription polymerase chain reaction (RT-PCR) from cDNA made from A549 human lung cancer cells. The PCR conditions were as follows: 95 °C for 3 min prior to the addition of Taq polymerase; 35 cycles of 94 °C for 40 s, 60 °C for 40 s, and 72 °C for 45 s; as well as a final extension of 72 °C for 10 min.

Purified PCR products were cut with HindIII/BamHI and inserted into the pCMV-Script vector (Promega, U.S.A.). Successful insertion of the PCR product was validated by HindIII/BamHI double digestion. The construct was sequenced by Boya Company (Shanghai, China). The plasmid was transformed into XL1-Blue competent cells, purified using a DNA extraction kit (Promega, U.S.A.), and validated by HindIII/BamHI double digestion.

RNA was extracted from cell monolayers grown to the exponential phase. Reverse transcription (RT)-PCR was performed according to the previously described method using the following primers: 5'-GAAAGCTTAAAGACAGAG-GAGGCT-3' and 5'-CCGGATCCAGATGCTTTTAGTT-3'. After gel electrophoresis, the integrated optical density of the amplified product (indicated by grayscale) was determined to measure the expression of BC006151, also known as lung cancer cell drug resistance gene. β-Actin was used as the internal control for RT-PCR, amplified with the following primers: 5'-ATCATGTTTGAGACCTTCAACA-3' and 5'-CATCTCTGTGCTGAAGTC-3'.

**Real-Time Polymerase Chain Reaction (PCR)** SYBR green I technology was used for the real-time PCR analysis. Total RNA was purified from tissues and cells as recommended by the manufacturer using Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). cDNA synthesis was performed using approximately 5 μg RNA per 20 μl using a cDNA reverse transcription kit (Fermentas). Real-time PCR was performed on an ABI 7500 system (Applied Biosystems). BC006151 primers were 5'-GACCTGGGCTCTTGCTCTCAAG-3' and 5'-TGGCTAGCTGACGGACACC-3'. The internal control β-actin primers were 5'-ATCATGTTTGAGACCTTCAACA-3' and 5'-CATCTCTGTGCTGAAGTC-3'. Primers were designed using Primer Express v3.0 Software. After first strand synthesis, an equivalent of 50 ng of starting total cellular RNA (1/10 of the cDNA reaction) was added to two duplicate PCR reactions containing 12.5 μl SybrGreen mix, 0.5 μl SybrGreen rox, 100 nmol/l forward primer, and 100 nmol/l reverse primer in a final volume of 25 μl. Each sample was used in a single reaction that cycled at 95 °C for 10 min (to activate enzyme), followed by 45 cycles of 95 °C for 10 s and 60 °C for 34 s on an ABI SDS 7500 system (Applied Biosystems). Fluorescent data were converted into cycle threshold measurements using the SDS system software. The 2^ΔΔCT method was used as a measure for the relative expression of the target gene. BC006151 mRNA levels were compared to actin. Thermal dissociation plots were examined for biphasic melting curves, indicative of whether primer-dimers or other nonspecific products could be contributing to the amplification signal.

**Cell Transfection** The drug-resistant H446/DDP SCLC cells were a generous gift from Dr. Linzhi Liu at Xinqiao Hospital of the Third Military Medical University. These cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO₂ in air. The H446/DDP SCLC cells transfected with the antisense expression vector using a lipid transfection method were named group T. The cells transfected with empty vector were named group C. Forty-eight hours after transfection, fresh RPMI-1640 medium with 10% FBS and G-418 (at a final concentration of 500 μg/ml) was added to the transfected cells.

**Cell Culture and Cell Proliferation Analysis** Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) medium supplemented with 10% FBS (Gibco/Invitrogen, NY, U.S.A.) in a humidified 37 °C incubator with 5% CO₂. Cells were grown in 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 1×10⁴ cells/ml. After 24, 48, 72, 96, or 120 h in culture, the numbers of drug-resistant H446/DDP cells and recombinant transfected cells were counted.

**MTT Assay to Determine the Chemosensitivity of H446/DDP Cells** The number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-y)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were cultured in 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 1×10⁴ cells/well, in the presence of cisplatin, etoposide, 5-fluorouracil, or topotecan. Each drug was applied at five different concentrations, increasing in a 5-fold gradient. The different chemotherapy drugs used for sensitivity test in vitro were 0.25, 0.5, 1, 2, 4 times of the dose for clinical plasma drug concentration, calculated according to the following formula: test drug concentration (mg/ml)=mg/average body surface area/average weight×100/60. After a 48-h incubation, MTT (Sigma, St. Louis, MO, U.S.A.), dissolved in phosphate buffered saline (PBS), was added to each well at a final concentration of 5 mg/ml, and the cells were then incubated at 37 °C for 4 h. The water-insoluble dark blue formazan crystals that form from MTT cleavage in actively metabolizing cells were dissolved in DMSO. The optical density was measured at a wavelength of 490 nm with a Bio-Rad 680 microplate reader (Bio-Rad, CA, U.S.A.). All experiments were performed in triplicate. The IC₅₀ (resistance index), i.e., 50% inhibitory concentration, of each drug was calculated from a logarithmic plot analysis. 50% inhibitory concentration=(1−ODdrug/ODcontrol)×100%.

**Transmission Electron Microscopy** H446/DDP cells were collected in the logarithmic growth phase. After the culture medium was removed, the cells were collected by scraping with a rubber rod and transferred to a 10 ml centrifuge tube. Pre-cooled PBS (4 °C) was then added, and the mixture was gently aspirated and dispersed until the cells were evenly distributed. After centrifugation at 2000 rpm for 15—20 min, the supernatant was discarded, and the tube was placed in an ice bath. Next, 30 ml/l pre-cooled glutaralde-
hyde (4 °C) was slowly added into the tube. After 30 min of fixation, cell clumps were carefully peeled, rinsed with PBS three times and fixed with 10 ml/l osmium tetroxide. After this fixation, the cells were rinsed with PBS, dehydrated, embedded, prepared on slides and stained with uranyl acetate before being subjected to transmission electron microscopy examination.\(^{(2)}\)

**Immunohistochemistry and Western Blot Analysis of Bel-2 Expression** Cells were trypsinized with 0.25% trypsin, plated on coverslips, incubated for 24 h, and then washed three times with Tris-buffered saline (TBS, Sigma). Cells were then fixed with 4% paraformaldehyde for 30 min, dried at 37 °C, washed three times with TBS, and equilibrated in TBS containing 0.3% Triton X-100 (Sigma). Endogenous peroxide activity was blocked by treatment with 0.3% H\(_2\)O\(_2\) (Sigma) in methanol for 30 min. Cells were then treated with 0.1% trypsin in 0.05 M Tris and 0.02 M CaCl\(_2\) (pH 8.0), and non-specific binding was eliminated by blocking with 1.5% normal goat serum (Santa Cruz Biotechnology, U.S.A.) and 0.5% bovine serum albumin (BSA) in TBS. The coverslips were incubated overnight at 4 °C with a primary antibody (rabbit polyclonal immunoglobulin G (IgG) to Bcl-2, diluted 1:200, Santa Cruz Biotechnology) in TBS with 0.5% BSA, followed by incubation with a secondary antibody (horse-radish peroxidase (HRP)-conjugated goat anti-rabbit IgG, Santa Cruz Biotechnology) and then with avidin/biotin complex (Santa Cruz Biotechnology) in TBS with 0.1% BSA for 1 h. Cells were developed with 0.5% 3,3′-diaminobenzidine (Sigma) in 0.1% H\(_2\)O\(_2\), 0.05 M Tris and 0.85% NaCl (pH 7.4) for 5 min, after which the coverslips were counterstained with Gill’s hematoxylin, dehydrated, and mounted with Permount (Sigma). A negative control in which the primary antibody was omitted, was used to verify the specificity of the immunohistochemical results.

Total cell protein was extracted in lysis buffer and centrifuged at 12000×g for 5 min at 4 °C.\(^{(1,2,11)}\) The supernatant was collected, and the total protein concentration was determined using Bio-Rad protein assay dye reagent (Bio-Rad). Aliquots (50 μg) of whole-cell lysates were loaded on sodium dodecyl sulfate-polyacrylamide (10%) gels for electrophoresis. For Western blot analysis, protein was transferred to Hybond ECL nitrocellulose membranes (Amersham Biosciences). The membranes were blocked with 5% nonfat milk in TBS containing 0.1% Tween-20 and incubated with the primary antibody (rabbit polyclonal IgG to Bcl-2, diluted 1:200, Santa Cruz Biotechnology) overnight at 4 °C, after which the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Promega) for at least 1 h at room temperature and detected by the ECL method (Amersham Biosciences).

**Flow Cytometry Analysis of Cell Cycle and Apoptosis** Cell cycle distribution and apoptosis were analyzed by flow cytometry as previously described.\(^{(2)}\) Briefly, following an incubation of 24, 48, 72, 96, or 120 h, cells were trypsinized with 0.25% trypsin (Sigma), counted, centrifuged at 300×g for 5 min and fixed in ethanol at 4 °C overnight. The cells were then washed and centrifuged. The resulting cell pellets were resuspended in a 0.02 mg/ml RNase solution (Sigma) containing 0.02 mg/ml propidium iodide (Sigma) and incubated at 4 °C for 30 min. The DNA content of approximately 1—2×10\(^5\) stained cells was analyzed by a FACSScan flow cytometer equipped with the FACStation data management system running CellQuest software (Becton-Dickinson, San Jose, CA, U.S.A.). Results are expressed as a logarithmic plot of fluorescence intensity versus cell number. Apoptotic rates=apoptotic cells/total cells×100%.

**Statistical Analysis** All statistical analyses were carried out using SPSS 14.0 statistical software. Data are expressed as mean±standard error of mean (S.E.M.) of individual experiments. Differences in the measured parameters among the groups were analyzed by one-factor ANOVA and the least significant difference test. Results with \(p\leq0.05\) were considered statistically significant.

**RESULTS**

**BC006151 Expression Is Elevated in Patient Tumor Tissues and SCLC Cell Line** RT-PCR was performed using the cDNA of the drug-resistant human lung adenocarcinoma cell line, A549/CDDP, as a template, and a 1298 bp target DNA fragment was obtained. As shown in Fig. 1A, we found that the BC006151 expression level was higher in the tumor tissues compared to that of the adjacent normal lung tissues. The BC006151 expression level in H446 cells was significantly decreased compared to that of drug-resistant H446/CDDP cells by real-time PCR (\(p<0.01\)) (Fig. 1B).

**Validation of the Constructed Recombinant pCMV-Script Vector and Integration of the BC006151 Antisense Gene in H446/CDDP Cells** After amplification, the recombinant plasmid was subjected to HindIII/BamHI double digestion, which generated a DNA fragment of 1298 bp, the expected length of the product, indicating that the BC006151 antisense gene was correctly inserted. The successful integration of the antisense gene into the genome of the transfected cells was validated by DNA sequencing. Gene sequencing was performed by Shanghai Boya Inc. (Fig. 1C).

**BC006151 Antisense Transfer Resulted in Reduced Expression of BC006151 mRNA and MDR Protein** The BC006151 expression level in the drug-resistant SCLC H446/CDDP cells was significantly decreased after 48 h transfected with the antisense gene, compared to that of the control cells transfected with empty vector (\(p<0.05\)) (Fig. 1D). The protein level of MDR, P-gp and GST-\(\pi\) were also decreased after 48 h transfected in the recombinant vector-transfected cells compared to controls (Figs. 1E, F). But, the trend of Topo II was reverse, and there was no significantly difference in the expression of GSH (Fig. 1F).

**Down-Regulation of BC006151 Caused a Reduction in Cell Proliferation** A reduction in cell number that became more pronounced over time was noted in the BC006151 antisense gene-transfected cells compared to the control cells. This difference reached statistical significance at 48 h (\(p<0.01\)) (Table 1).

**Down-Regulation of BC006151 Made Lung Cancer Cells Less Resistant to Chemotherapy** The MTT method was used to measure the cell optical density 48 h after transfection. The resistance index for each drug was significantly decreased in the antisense gene-transfected cells compared to that of controls (\(p<0.01\)) (Table 2). In each chemotherapy group, the expression of MDR was decreased after 48 h transfected with the antisense gene, compared to that of the control cells transfected with empty vector (Fig. 1G).
DISCUSSION

H446/DDP is a cisplatin-induced drug-resistant cell line generated from H446 cells that is resistant to many chemotherapeutic agents. In this study, we showed by MTT assay that these MDR-expressing H446/DDP cells were resistant to several chemotherapeutic agents that have different pharmacological effects and are frequently used in the clinic, including cisplatin and etoposide. Flow cytometry results confirmed that there was no significant difference in cell cycle phase distribution between H446 and H446/DDP cells, reducing potential error in drug resistance detection. The H446/DDP cell line is therefore suitable for in vitro MDR study.

**BC006151 Antisense Gene Transfection Increased Cellular Apoptosis** Apoptosis was detected in the antisense gene-transfected H446/DDP cells by transmission electron microscopy, evidenced by the observation of increased intracellular vacuoles, a large number of lamellary bodies, swollen mitochondria and small pieces of cytoplasm shed from cells; significantly decreased cell volume; and apoptotic bodies, i.e., cytoplasmic fragments containing chromatin and organelles shed from apoptotic cells (Fig. 2A). In contrast, no apoptosis was detected in the empty vector-transfected cells.

**Down-Regulation of BC006151 Altered Cell Cycle Distribution and Apoptosis** Flow cytometry was used to determine the cell cycle phase distribution. The percentage of G1-phase cells in the antisense gene-transfected group (G1 phase, 76%; S phase, 13%) was significantly higher than that of the control group (G1 phase, 56%; S phase, 23%) (p < 0.01) (Fig. 2B). Apoptotic rates were measured by double-staining flow cytometry analysis. The apoptotic rate of the antisense gene-transfected group was 13.85 ± 1.79%, significantly higher than that of the control group, 3.21 ± 0.52% (p < 0.01) (Fig. 3A).

**Bel-2 Expression Was Reduced in BC006151 Antisense-Transfected Cells** Expression of an apoptosis inhibitor, Bel-2, was evaluated by immunohistochemistry and Western blot. As shown by the immunohistochemical results, Bel-2 was present in the cytoplasm of resistant SCLC H446/DDP cells, and its expression was significantly inhibited in the antisense gene-transfected cells compared to controls (Fig. 3B). Similar results were obtained by Western blot analysis (Fig. 3C).

**Table 1. Cell Count of H446/DDP at Different Time-Points**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>C</th>
<th>T</th>
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<tr>
<td>0</td>
<td>1.08 ± 0.05</td>
<td>1.02 ± 0.03</td>
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<tr>
<td>24</td>
<td>1.55 ± 0.19</td>
<td>1.38 ± 0.16</td>
</tr>
<tr>
<td>48</td>
<td>2.52 ± 0.31</td>
<td>1.76 ± 0.29*</td>
</tr>
<tr>
<td>72</td>
<td>5.23 ± 0.48</td>
<td>3.39 ± 0.35*</td>
</tr>
<tr>
<td>96</td>
<td>8.78 ± 0.73</td>
<td>5.28 ± 0.61*</td>
</tr>
<tr>
<td>120</td>
<td>12.99 ± 0.96</td>
<td>7.84 ± 0.81*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group C (μg/ml)</th>
<th>Group T (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>0.7653 ± 0.059</td>
<td>0.3092 ± 0.032*</td>
</tr>
<tr>
<td>Etoposide</td>
<td>0.5335 ± 0.061</td>
<td>0.2458 ± 0.029*</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>2.3591 ± 0.317</td>
<td>1.1657 ± 0.157*</td>
</tr>
<tr>
<td>Topotecan</td>
<td>0.6516 ± 0.052</td>
<td>0.3126 ± 0.036*</td>
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Drug resistance indexes of H446/DDP cell to cisplatin, etoposide, 5-fluorouracil, and topotecan after transfected BC006151 antisense gene were determined respectively. The values are shown as the mean ± S.E.M. *p < 0.01 compared to the control group [least significant difference (LSD) test by ANOVA].

**Table 2. Multi-Drug Resistance Index of H446/DDP Cell after Transfected BC006151 Antisense Gene**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group C</th>
<th>Group T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>0.036*</td>
<td>0.157*</td>
</tr>
<tr>
<td>Etoposide</td>
<td>0.029*</td>
<td>0.032*</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>0.036*</td>
<td>0.029*</td>
</tr>
<tr>
<td>Topotecan</td>
<td>0.036*</td>
<td>0.029*</td>
</tr>
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</table>

Drug resistance indexes of H446/DDP cell to cisplatin, etoposide, 5-fluorouracil, and topotecan after transfected BC006151 antisense gene were determined respectively. The values are shown as the mean ± S.E.M. *p < 0.01 compared to the control group [least significant difference (LSD) test by ANOVA].
The SSH method is a useful approach for cloning differentially expressed genes. Because of its high specificity, low false positive rate and the ability to detect low-abundance mRNA, this method has been widely accepted and applied in the field. In a previous study, we obtained a 494-bp cDNA fragment from a lung cancer MDR cell line, SPC-1/CDDP, by the SSH method. Homology analysis using the GenBank database revealed a 99% homology between this sequence and the sequence of a 1316-bp full-length new mRNA transcript, BC006151. This mRNA transcript was reported in April 2001 and named lung cancer cell drug resistance gene (LCDRG), although its function has not been characterized.

We amplified a 1298-bp fragment of this gene by RT-PCR, and the sequencing result indicated that the original 494-bp fragment was part of this BC006151 gene. We also showed that the BC006151 expression level in H446/CDDP cells was significantly higher than that of the parental cell line, H446, indicating that this gene is closely related to CDDP-induced resistance in lung cancer.

In recent years, some oncogene products associated with tumor initiation, development and recurrence have been found to be closely related to drug resistance in tumor cells. These drug resistance mechanisms, individually or in combination, may play particular roles in MDR. The development of MDR in lung cancer results from the combined effects of multiple genes. Antineoplastic agents induce apoptosis, while the cytotoxicity of chemotherapeutic agents is mainly exerted by triggering programmed cell death pathways in tumor cells. Apoptosis is likely the shared final pathway for the effects of most chemotherapeutic agents. Drug resistance of tumor cells usually results from the inhibition of cell apoptosis. So, alterations in susceptibility to apoptosis not only contribute to neoplastic development, but also can enhance resistance to conventional anticancer therapies.

One of the suggested mechanisms of resistance to cytotoxic antineoplastic drugs is the alteration in expression of B-cell lymphoma-2 (Bcl-2) family members. The Bcl-2 family
of proteins consists of 25 pro- and anti-apoptotic members, which interact to maintain a balance between newly forming cells and old dying cells.19—22) When anti-apoptotic Bcl-2 family members are overexpressed, the ratio of pro- and anti-apoptotic Bcl-2 family members is disturbed and apoptotic cell death can be prevented. Targeting the anti-apoptotic Bcl-2 family of proteins can improve apoptosis and thus overcome drug resistance to cancer chemotherapy.

Unlike most oncoproteins that promote proliferation, Bcl-2 functions by preventing programmed cell death. As the anti-apoptotic Bcl-2 family proteins promote cancer cell survival by antagonizing apoptosis, they provide therapeutic targets, and inhibition of anti-apoptotic Bcl-2 family proteins is expected to predominantly induce apoptosis in cancer cells. Bcl-2 was identified because of a characteristic chromosomal translocation t(14;18) present in 85% of follicular lymphomas and 20% of diffuse B-cell lymphomas, which results in deregulated Bcl-2 gene expression at the transcriptional level. The in vivo effects of Bcl-2 were initially investigated in Bcl-2 transgenic mice in which Bcl-2 overexpression was targeted to follicular hyperplasia or T-cell lymphomas. Constitutively high levels of Bcl-2 or Bcl-XL have been associated with a more aggressive malignant phenotype and/or drug resistance to various categories of chemotherapeutic agents in hematologic malignancies and solid tumors. It has been speculated that the balance between anti- and pro-apoptotic Bcl-2 family members, rather than mere overexpression of Bcl-2, regulates the death of cancer cells.22,23)

It had been confirmed that the upregulation of Bcl-2 expression is significantly correlated with the inhibition of apoptosis induced by various chemotherapeutic agents. In most small cell lung cancer (SCLC) tumor samples and cell lines, Bcl-2 upregulation is correlated with tumor resistance to radiotherapy and chemotherapy.13,24—26) Application of Bcl-2 antisense oligonucleotide can promote apoptosis in SCLC cells, and it also displays synergistic cytotoxic effects with etoposide, doxorubicin and cisplatin.27,28) Additionally, it has been reported that Bcl-XL is a more important prognostic indicator in adenocarcinoma than in SCLC, but that Bcl-XL antisense oligonucleotide treatment has therapeutic effects on most lung cancers.29) From the present study, we think that BC006151 gene regulating the expression of MDR proteins though bcl-2 family gene mediated, some protein such as P-glycoprotein (P-gp), topoisomerase (Topo II), and glutathione GSH-S-transferase (GST) may be involved in the regulation. But the detail signal pathways need more work to be confirmed.

In summary, we successfully cloned a cDNA fragment of BC006151, whose expression was significantly increased during the development of CDDP-induced drug resistance in the human lung cancer cell line, H446/CDDP. BC006151 antisense gene transfection increased chemosensitivity, inhibited cell proliferation and significantly inhibited Bcl-2 gene expression in H446/CDDP cells, indicating that this treatment might inhibit tumor cell proliferation and enhance cellular apoptosis. This study provided an empirical basis for further investigation into the biological functions of BC006151, a new lung cancer drug resistance-related gene, and it encourages future efforts to elucidate the mechanism by which it regulates the development of lung cancer MDR.

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