Effects of gemcitabine and cisplatin chemotherapy in advanced non-small cell lung cancer patients with RRM1 low protein expression

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Abstract  **Objective:** The aim of this study was to observe the efficacy of gemcitabine combined with cisplatin (GP) in advanced non-small cell lung cancer (NSCLC) patients with low expression of ribonucleotide reductase 1 (RRM1) protein using immunohistochemistry.  **Methods:** RRM1 protein expression in tumor tissue was detected by streptavidin-peroxidase (SP) method of immunohistochemistry. GP regimen (gemcitabine 1000–1250 mg d1, d8, cisplatin 75 mg/m²) was given to advanced NSCLC patients with low expression of RRM1 protein.  **Results:** In the total of 40 patients, these patients with RRM1 low expression performing GP chemotherapy had a good response rate, the objective response rate (ORR) was 47.5% (95% CI, 32.02%–62.98%), and the disease control rate (DCR) was 72.5% (95% CI, 65.44%–79.56%). ORR is 45.45% (5/11) in the squamous cell carcinoma patients while 48.15% (13/27) in the adenocarcinoma patients.  **Conclusion:** Superior ORR and DCR were found in advanced NSCLC patients with low expression of RRM1 protein expression performing GP regimen.

Key words  gemcitabine; ribonucleotide reductase 1 (RRM1); immunohistochemistry; chemotherapy; non-small cell lung cancer (NSCLC)

80%–85% of lung cancer cases, as one of the most common malignant tumors in the world, are non-small cell lung cancer (NSCLC). At present, the platinum-based chemotherapy with the third-generation chemotherapeutic drugs is the standard front-line chemotherapy in advanced NSCLC. Among them, the third-generation chemotherapeutic drugs (paclitaxel, docetaxel, gemcitabine and vinorelbine) with the objective response rate (ORR) of 17%–22% [(1, 2)] showed no significant difference in the overall treatment effect of advanced NSCLC, and the ORR was reported to be 30%–45% [(3, 4)] among Chinese people. Therefore, there is no definite guiding principle for the choice of third-generation chemotherapeutic drugs. Nevertheless, if there is an effective biomarker which can help to choose chemotherapeutic drugs, not only will the treatment effect of chemotherapy be improved, but also the undesired toxic side effect can be avoided. In the recent years, various drug-sensitive biomarkers such as ribonucleotide reductase 1 (RRM1) have been extensively studied, and it is generally believed that the high expression of RRM1 mRNA in tumor tissues is associated with gemcitabine resistance [(5–7)], as mRNA of tumor tissues has certain correlation with protein expression. Some scholars have also found through retrospective analyses that the patients with lower RRM1 protein expression receiving gemcitabine chemotherapy showed longer median and overall survival time [(8, 9)] that the patients with high expression. To further investigate the therapeutic effect of using gemcitabine and cisplatin (GP) as the first line treatment in the advanced NSCLC patients with low RRM1 expression, the semiquantitative immunohistochemical method was employed in our study to detect RRM1 protein expression in tumor tissues of advanced NSCLC, and the patients with lower RRM1 expression were selected to receive GP therapy and follow-up visit.
Materials and methods

Research design and inclusion criteria

A total of 40 cases from October of 2009 to August of 2010 were included. The inclusion criteria were as follows: (1) NSCLC patients pathologically diagnosed at advanced stage who were unable to receive operation; (2) there were measurable lesions; (3) tumor specimens were acquired at the initial diagnosis when the patients had not received anti-tumor treatments such as operation and radiotherapy & chemotherapy; (4) patients with tumor specimens detected by immunohistochemistry of RRM1 protein to low expression; (5) first-treated patients; (6) the PS scoring of the patient was 0 or 1; (7) the major organs of the patients functioned normally.

Determination and judgment by immunohistochemistry

Immunohistochemistry-SP (streptavidin-peroxidase) method was adopted to detect RRM1 expression in pathological section. Human anti-murine RRM1 polyclonal antibody, SP kit and DAB color developing reagent were all purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (China). The tissue specimens obtained were manufacture into paraffin sections with the thickness of 4 μm. After dewaxed and hydrated, they were subject to pretreatment. Next, the tissue sections were cultured in 3% H2O2 solution at room temperature for 10 min, and then washed by distilled water for three times, 2 min for each time. After that, 1:100 diluted anti-RRM1 polyclonal antibodies were added into these tissue sections, which were then cultured at room temperature for 20 min. The following experimental steps included DAB color development, staining with hematoxylin for comparison, dehydration, vitrification by dimethylbenzene, and sealing with neutral resins in sequence. At last, using PBS as a substitute of primary antibody for negative control and the known positive section as a positive control, it was observed under microscope.

Assessment criteria: the appearance of the yellowish brown particles in tumor cell nuclei was set as positive. The criteria for immunohistochemistry: at 400 magnification times, 500–1500 tumor cells were selected randomly from each specimen for the observation of the intensity of positive cellular staining, and the number of positive cells was counted. The scoring of the intensity of cellular staining ranged between 0–3 points: no staining was scored as 0 points; pale yellow staining 1 point; yellowish brown staining 2 points; dark brown or umber brown staining 3 points. The scoring range for the constituent ratio of positive cells together, the total score of 0–1 points was defined as negative (−) and 2 or more points positive (+).

Treatment

Prior to treatment, the white blood cell count and platelet count of each patient was above 4 × 10^9/L and 100 × 10^9/L, respectively, and the liver, kidney and heart functioned normally for all of the patients. Chemotherapy: all of the patients were given 1000–1250 mg/m² intravenous infusion of gemcitabine (Gemzar, Eli Lilly, USA) according to the surface area of body on the first day and on the eighth day, respectively. 75 mg/m² intravenous infusion of cisplatin was conducted on a total of three days. Each intravenous infusion of Gemzar lasted for 30 min; before chemotherapy, pre-treatment was conducted with dexamethasone; during chemotherapy, 5-HT3 receptor antagonist was used as antiemetic drug. The cycle of this treatment lasted for 21 days, and the patients who had received at least 2 cycles of this treatment were subject to curative effect assessment.

Assessment of curative effect

There were measurable lesions for all of the cases included, and the comparison was made with the results of thin-slice spiral CT ± enhanced scanning in our hospital. Response rates of all patients were assessed according to RECIST standards. Complete remission (CR); partial remission (PR); stabilization of disease (SD); progress of disease (PD); objective remission rate (ORR) included CR and PR confirmed after at least four weeks. Disease control rate (DCR) included the confirmed cases with tumor remission (CR and PR) and the cases with SD after at least 6 weeks. All of the patients were subject to follow-up visit once every month until the change of treatment, progress of disease, death or loss of follow-up.

Statistical analysis

SPSS 13.0 software was adopted for statistical processing. 95% confidence intervals (CI) of effectiveness rate were subject to statistical process.

Results

Data of the patients

A total of 40 cases were included, with the details shown in Table 1; 29 were male patients, and 11 were female patients, with the age ranging between 37–80; the median age was 59 (59.4 ± 10.9, χ ± SD); 18 cases were ≥ 60, and 22 cases were < 60. The tumor specimens of 20 cases were obtained by centesis with bronchosfiberscope; 11 cases by percutaneous lung biopsy; 9 cases by supraclavicular node biopsy or cervical lymph node biopsy. 11 cases were diagnosed as squamous carcinoma, 27 cases adenocarcinoma, and 2 cases large cell carcinoma. All of