Clinical Observations

THE CORRELATION BETWEEN THE EXPRESSION OF MULTIDRUG RESISTANCE RELATED GENE AND CELL APOPTOSIS AND CLINICAL SIGNIFICANCE IN NON-SMALL CELL LUNG CANCER

WANG Jie, LIU Xu-yi, LI Xi-ping, JIANG Wei, LIANG Li

Department of Medical Oncology, The School of Oncology, Peking University, Beijing 100036, China

ABSTRACT

Objective: To explore the correlation and clinical significance between expression of MDR (multidrug resistance) related gene MRP, MDRI, C-erbB-2 and cell apoptosis in non-small cell lung cancer (NSCLC).

Methods: RT-PCR, Immunohistochemistry were used to examine the expression of mRNA and protein in the MDR and apoptosis related gene. Apoptosis cells were assayed by Terminal deoxynucleotidyl transferase (TdT)- mediated biotin dUTP nick end-labeling (TUNEL). Results: The positive rates of MRP, MDRI, C-erbB-2, bcl-2, C-myc mRNA in 63 cases NSCLC were 81.0% (51/63), 38.1% (24/63), 47.6% (30/63), 65.1% (41/63), 76.2% (48/63) respectively. Their levels were higher than those of corresponding proteins (74.6%, 34.9%, 46.0%, 61.9%, 71.4%, respectively). The significant association was found between the mRNA level and the protein expression (r = 0.764, P<0.02). The C-myc expression in 2 cases adjacent and benign lung tissue were light positive, and another 3 cases were negative. The positive correlation were demonstrated between C-myc and C-erbB-2 (r=0.547, p=0.001) as well as bcl-2 and C-erbB-2 (r=0.486, p=0.023) in NSCLC. There is no any correlation among C-myc and MRP or MDRI. There exists inverse correlation between apoptotic index and bcl-2 (r = -0.587, p = 0.017), and no any correlation among apoptotic index and MRP or MDRI or C-erbB-2 or C-myc. The average apoptotic index were higher in the effective chemotherapy group (27.2±2.1, 30.5±1.8) than that in the non-effective chemotherapy group (9.4±1.3, 12.6±2.4) with adenocarcinoma and squamous cell carcinoma (p =0.01, p=0.004). The positive rates of bcl-2, MRP, C-erbB-2 expression in the effective chemotherapy group (31.8%, 40.9%, 22.7%, respectively) were lower than those in the non-effective chemotherapy group (77.4%, 90.3%, 67.7%, respectively) (p=0.036, p=0.012, p=0.01), but MDRI, and C-myc expression have no any significant difference (p=0.067, p=0.282). The median survival time in the patients with coexpression of more than three MDR and/or apoptosis related genes are shorter (8.6 months) than that in those patients with coexpression of less than three MDR and/or apoptosis related genes (15.5 months) (p=0.01). Conclusion: The multidrug resistance in NSCLC is not only related to many drug resistance genes, but also involved in cell apoptosis and apoptosis related gene expression. The coexpression of MDR and apoptosis related gene is related to the survival time.

Key words: Lung cancer, Multidrug resistance, Apoptosis, Related gene

The mechanisms about multidrug resistance (MDR) which are studied widely in present include overexpression of multidrug resistance protein such as MDR1/P-gp, MRP (multidrug resistance related protein), LRP (lung resistance protein), increased detoxification of Glutathione / Glutathione - S - transferase II. Recent studies have showed that inhibition of cell apoptosis and overexpression of apoptosis related gene is another reason for the MDR. But there are few reports about the correlation between the expression of MDR related gene and cell apoptosis and their prognostic
significant in lung cancer tissues. In our experiment, the techniques of RT-PCR, Immunohistochemistry, Terminal deoxynucleotidyl transferase (TdT)-mediated biotin dUTP nick end labeling (TUNEL) of apoptotic cells assay were used to explore the correlation among the expression of MDR and apoptosis related gene in the frozen lung cancer tissues as well as the relation with response to chemotherapy and prognosis.

MATERIALS AND METHODS

Patients and Tumor Samples

Specimens from 63 patients with non-small cell lung cancer untreated with chemotherapy were obtained by biopsing of metastatic lumps outside the thorax or undergoing surgery for primary tumors. The tissues were immediately frozen and stored in liquid nitrogen for use. 21 patients were female and 42 were male. The median age of the patients (at time of biopsy or surgery) was 56.5 years (range 17-74). The tumor-node-metastasis (TNM) stage was 6 in stage I, 12 in stage II, 6 in stage IIIa, 16 in stage IIIb, and 23 in stage IV, respectively. The 53 patients were treated with chemotherapy after biopsied or operated surgery (at least 2-3 cycles). The main chemotherapies were asplatin based. The response was evaluated according to the WHO’s criterion. Those who got to the complete respond (CR) or partial respond (PR) for at least four weeks were effective to chemotherapy. The 22 cases were response to the treatment (13 were adenocarcinoma and 9 were squamous cell carcinomas), 31 cases were no effective. The median follow-up duration was 31 months (1-77 months). 38 died during follow-up. All were contrasted with the adjacent and benign lung tissue.

RNA Isolation and RT-PCR

Frozen samples 50mg stored in the fluid nitrogen were homogenized in 100μl 0.01 mol/L PBS buffers. Total RNA was extracted and RT-PCR was performed as previously described.[5] Every target gene primer and the annealing temperature was seen in the Table 1. 10 μl each polymerase chain reaction (PCR) product was separated on a 1.8% agarose gel in TBE at 150 v for 1 h after staining with 0.5 μg/ml ethidium bromide. The amplification product was then scanned by a Molecular Dynamics Densitometer (Sunnyvale, CA) and got A value. Relative quantity of target gene mRNA was calculated by the ratio of A value of target gene and β-acting.

Table 1. Primer sequences of target genes and PCR reaction condition

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence</th>
<th>T</th>
<th>N</th>
<th>Amplification product</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP</td>
<td>S TCTTCCCGACATGACCGAGG As CCAGGAATATGCCCCGACTTC</td>
<td>55</td>
<td>29</td>
<td>291</td>
</tr>
<tr>
<td>MDR1</td>
<td>S CCCATCATTTGCAATAGCAGG As GTTCAAAACTTCTGCTCTTAG</td>
<td>55</td>
<td>29</td>
<td>167</td>
</tr>
<tr>
<td>C-erbB-2</td>
<td>S CCCACGTCGGTAGAAGGTA As TGAACAAATCCACCCTGTC</td>
<td>55</td>
<td>30</td>
<td>240</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>S CAGCAGTCTCCTCGCGGTACCCGC As CGGCTAGCTGGGGCCGTACAGTTC</td>
<td>58</td>
<td>35</td>
<td>318</td>
</tr>
<tr>
<td>C-myc</td>
<td>S ATTCTCTGCTCTCTGGAC As TCCAGACTCTGCCTTGTGC</td>
<td>55</td>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>β-actin</td>
<td>S AGCATCCTAGAACTCTGTGC As ATTTCCGACCCCTGAACATA</td>
<td>55</td>
<td>29</td>
<td>400</td>
</tr>
</tbody>
</table>

T: annealing temperature, N: Cycle times

Apoptotic Cells were Evaluated by TUNEL Assay

The apoptotic cells were detected by TUNEL as described.[6] The peripheral blood lymphocytes treated with dexamethazone was as positive control. The adjacent and benign lung tissues were as negative control. The apoptotic index (percentages of apoptotic cells among tumor cells) was determined by comparing the number of apoptotic cells and viable tumor cells in 5 HPFs.