SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 11: 1413-1417, 2016

Effect of hydrothorax EGFR gene mutation and EGFR-TKI targeted therapy on advanced non-small cell lung cancer patients

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Received September 23, 2015; Accepted December 30, 2015

DOI: 10.3892/ol.2015.4066

Abstract. Lung cancer is a malignancy with the highest incidence of morbidity and mortality worldwide. The lack of effective detection methods leads to the ineffectiveness of convetional therapy. The aim of the current study was to analyze the hydrothorax epidermal growth factor receptor (EGFR) mutation in patients with advanced non-small lung cancer (NSCLC) and malignant pleural effusion. A new method for clinical treatment was developed through a comparison of the difference of EGFR tyrosine kinase inhibitor (EGFR-TKI)-targeted therapy. Between January 2013 and January 2015, 68 cases diagnosed with advanced non-small lung cancer and malignant pleural effusion, were enrolled in the study. Previous first-line chemotherapeutic treatment schemes had been unsuccessful. EGFR 19 and EGFR 21 sites were detected for all the patients. Platinum-based drugs were provided for patients with wild-type EGFR. These patients served as the control group and underwent four cycles of treatments, with each cycle lasting 3 weeks. TKI medicine Gefitinib (Iressa™) was administered to patients with mutant EGFR tid, po, for a duration of 4-8 months. These patients served as the experimental group. There were 41 cases of EGFR mutations, of which 13 cases had EGFR 19 site mutations, 16 cases EGFR 21 site mutations, and the remaining 12 cases had 2 site mutations. EGFR mutations were not significant for gender, age, tumor type, stage and diameter (P>0.05). The results showed that the six-month survival rate, progression-free survival time (PFS), objective response rate (RP) and disease control rate (DCR) in the experimental group were higher than those in the control group. The drug side-effects in the experimental group indicated no statistical differences compared to the control group (P>0.05). The incidence of EGFR mutation was higher in patients with advanced non-small lung cancer and malignant pleural effusion. Targeted therapy improved the survival rate and was deemed to be a safe and effective method for patients with EGFR mutations.

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Key words: advanced non-small lung cancer patients, epidermal growth factor receptor, mutation, targeted therapy, gefitinib

Introduction

Lung cancer is a leading cause of cancer worldwide, with a high morbidity and mortality rate. Available data show that non-small lung cancer (NSCLC) accounts for 80-85% of all lung cancers (1). Early clinical manifestation of NSCLC in patients is not typical, and the lack of sensitive and effective detection methods reduces the opportunity for early detection of this disease. The majority of lung cancer patients are diagnosed at advanced stages when surgery is no longer a viable option. Conventional chemotherapy is shown to be ineffective for these patients (2). Advances in cellular and molecular biology and development of new molecular-targeted drugs with clinical applications lead to a higher survival rate of patients with advanced NSCLC (3). Drugs known as tyrosine kinase inhibitors (TKIs) are now considered a standard treatment for patients with epidermal growth factor receptor (EGFR) mutations (2). The results of previous studies on the efficiency of targeted therapy as an independent treatment for advanced NSCLC patients, short of application of surgery or chemotherapy, are inconsistent. There are also conflicting reports on whether EGFR mutation patients are sensitive to this type of treatment (5,6).

The aim of the present study was to identify hydrothorax EGFR mutations in patients with advanced NSCLC and malignant pleural effusion. The differences in EGFR-TKIs-targeted therapy effects between the control and experimental groups were compared and a new method for the clinical treatment was subsequently identified.

Materials and methods

General materials. Between January 2013 and January 2015, 68 cases diagnosed with advanced NSCLC and malignant pleural effusion, were enrolled in the present study. The subjects comprised 41 males and 27 females, aged 46-75, with

an average age of 59.7±11.6 years. Fourteen cases underwent surgery and 33 patients were treated by chemotherapy. All the cases were patients from The First People's Hospital of Yichang (Hubei, China) and any previous attempts to treat these patients using first-line chemotherapeutic schemes were unsuccessful.

Patients were included in the study based on the following criteria: i) Patients were between 18 and 80 years of age; ii) patients were confirmed cases through treatments including surgery, hydrothorax specimen pathology and CT; iii) patients had a KPS score >60 points. The exclusion criteria for the study were: i) Patients with secondary lung tumor combined with tuberculosis, tuberculous pleural effusion and other types of tumors were excluded; ii) parturient patients, patients previously treated with chemotherapy drugs, those with allergy or intolerance, and cases with infection as well as autoimmune disease were excluded; iii) patients with severe heart, liver, kidney and other viscera dysfunction, patients with serious coagulation disorders and patients with a life expectancy period <1 year; and iv) cases with poor compliance, patients with severe mental disorders and those refusing to participate in this study were excluded.

Methods. Approval from the ethics committee of The First People's Hospital of Yichang was obtained. Written informed consent was obtained from all patients and their families.

EGFR 19 and 21 sites were detected in all the cases participating in this study. Platinum-based drugs were administered to patients with wild-type EGFR in control group. Patients were subjected to four cycles of treatments and each cycle lasted 3 weeks. TKI medicine-Gefitinib (Iressa™) was administered to patients with mutant EGFR (as experimental group), tid, po, for a duration of 4-8 months.

Routine blood examinations, heart, liver and kidney functions and coagulation indicators were monitored periodically. Fever, white blood cell reduction and other complications were treated symptomatically.

Detection methods for hydrothorax EGFR mutations were subsequently carried out. Briefly, B-ultrasound localization was used to carry out the chest catheter closed drainage for patients, 50 ml hydrothorax was extracted and centrifuged at 3,000 x g for 15 min and subsequently the pellets were embedded with paraffin. The DNA FFPE Tissue kit (Qiagen, Valencia, CA, USA) was employed to extract DNA samples according to the manufacturer's instructions. Agarose gel (1.2%) was used for DNA identification, and the Q-3000 trace ultraviolet spectrophotometer (Quell Technology, Waltham, MA, USA) was used for DNA content detection. PCR was used for amplification and sequencing of EGFR 19 and 21 exons. ABI Sequencing Analysis v 5.4 software (Applied Biosystems, Foster City, CA, USA) was applied to analyze the sequencing results to identify the EGFR mutations. A search was conducted for the following mutations: i) EGFR exon 19 deletions, ii) L858R mutation (amino acid substitution at position 858 in EGFR, from a leucine to an arginine) in exon 21, iii) the L826Q mutation (amino acid substitution at position 861 in EGFR, from an isoleucine to a Serine) in exon 21.

Observational indices. General data from the two groups were compared including, differences between gender, age, tumor type, stage of the tumor, diameter of the tumor, 6-month survival rate, progression-free survival time (PFS), objective response rate (RP), disease control rate (DCR) and drug side-effects. PFS referred to the time span from entering the group to tumor progression or death. According to the Response Evaluation Criteria in Solid Tumors, the effective evaluation criteria were divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The PR was a percentage of CR+PR, and the DCR was a percentage of CR+PR+SD. According to the Common Terminology Criteria for Adverse Events (the 3rd edition) created by the American National Cancer Institute (NCI), drug side-effects such as contained hematology, general situation, skin system, digestive tract, liver, urinary system, cardiopulmonary, and blood vessel nervous system were considered.

Statistical analysis. SPSS 2.0 software (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Measurement data were presented with mean ± SD, and comparisons between groups were performed using the t-test. Case numbers or percentages were used to express count data. The χ^2 test was used for comparison between groups. $P<0.05$ was considered statistically significant.

Results

Comparison of general data between two groups. A total of 41 cases were identified with EGFR mutations (60.29%). Of these, 13 cases had EGFR 19 site mutation, 16 had EGFR 21 site mutation, and the remaining 12 had 2 site mutations. Presence or absence of EGFR mutations did not correlate with gender, age, tumor type, stage and diameter ($P>0.05$; Table I).

Comparison of the 6-month survival rate, PFS, objective response rate and disease control rate between the two groups. The 6-month survival rate was 51.2%, PFS was 5.7 months, the objective response rate was 56.1% and the disease control rate was 58.5% in the experimental group, which were significantly higher than those in the control group. The differences identified were statistically significant ($P<0.05$; Table II).

Comparison of drug side-effects between the two groups. The drug side-effects (17.1%) in the experimental group were compared to the control group (18.5%), and the differences were not statistically significant ($P<0.05$; Table III).

Discussion

Molecular-targeted therapy refers to a type of cancer treatment considered to treat malignant tumors by interfering with molecular irregularities that stimulate tumor growth (7). This type of treatment can block or interfere with a specific biochemical pathway that is central to the development, growth and spread of cancer such as the cell signal transduction pathway, the original balance of oncogenes and tumor suppressor genes and tumor angiogenesis (8).

The method is expected to prevent or reverse the malignant behaviors of normal cells and suppress tumor growth,

Table I. Comparison of general data between two groups.

Group	Cases	M/F	Age	Adenocarcinoma	Squamous carcinoma	Stage III	Stage IV	Diameter (cm)
Control group	27	19/8	60.4±12.7	15	12	16	11	3.4±1.1
Experimental group	41	22/19	61.7±13.2	23	18	24	17	3.6±1.3
t- and χ^2 tests		0.527	0.129	0.227	0.649	0.926		
P-value		0.326	0.413	0.832	0.327	0.728		

Table II. Comparison of 6-month survival rate, PFS, objective response rate and disease control rate between the two groups.

Group	Cases	CR	PR	SD	PD	Six-month survival rate	PFS (months)	RR	DCR
Control group	27	3	5	1	18	7 (25.9)	4.5±0.8	8 (29.6)	9 (33.3)
Experimental group	41	9	14	1	17	21 (51.2)	5.7±1.2	23 (56.1)	24 (58.5)
t- and χ^2 tests						4.300	5.267	4.598	4.140
P-value						0.038	0.024	0.032	0.042

PFS, progression-free survival time; CR, complete response; PR, response rate; SD, stable disease; PD, progressive disease; DCR, disease control rate.

Table III. Comparison of drug side-effects between two groups (%).

Group	Cases	Bone marrow transplantation	Digestive tract symptom	Liver and kidney lesions	Adverse reaction incidence
Control group	27	2	2	1	5 (18.5)
Experimental group	41	3	2	2	7 (17.1)
χ^2					0.637
P-value					0.259

recurrence and metastasis (9). Compared with traditional chemotherapy drugs, these drugs have characteristics of targeting without cytotoxicity. Molecular-targeted drug treatment has the advantages of high efficiency and low toxicity which are valuable qualities absent in conventional chemotherapy. This method of cancer therapy opens up a new field of molecular/biological treatment (10). Molecular-targeted therapy is gradually gaining momentum, and has become one of the most promising methods and strategies for lung cancer treatment in the 21st century (10).

The most thorough study of targeted therapy concerns the tyrosine kinase inhibitor in EGFR signaling pathway (11). EGFR belongs to the type I growth factor family and is the expression product of oncogene C-erB-1 (HER-1), located on the cell membrane. EGFR mutations of NSCLC are >90% and are found in the exons of chromosomes 19 and 21 (12). EGFR is expressed in epithelium, mesenchyme and neurogenic organization. It is important in the proliferation, growth and differentiation of normal cells. EGFR is also closely associated with the growth of tumor cells, angiopoiesis, tumor metastasis and inhibition of cell apoptosis (13).

Ligands combine with the N-terminal extracellular domain of EGFR forming homogenous or heterogenous dimers. Phosphorylation of intracellular tyrosine residues activates downstream signaling pathways, including the RAS/RAF/ERK/MAPK, P13K/AKT, STAT3/5 pathways. Consequently, a series of abnormal biological behaviors of tumor cells such as proliferation, invasion and metastasis, angiogenesis or disorder, and promotion of cell dysplasia manifest themselves (14). Dimers formed on the N-terminal extracellular domain and ligands are the premise of the entire signaling pathway, and play a key role in the phosphorylation of tyrosine residues in EGFR. The mechanism of TKI drugs such as gefitinib, erlotinib and icotinib is competitive by binding with the ATP binding site located in the EGFR intracellular tyrosine kinase, and arresting the conduction of EGFR downstream signaling pathways in order to inhibit or kill the tumor cells (15). Mutations, by changing the conformation of the tyrosine kinase domain of EGFR result in TKI drugs combining readily with EGFR and enhancing the sensitivity of TKI drugs (16). These phenomena stimulate EGFR mutations amd may be used as predictive indices for the judgment

of TKI drug efficiency. A number of basic and clinical investigations showed that the majority of EGFR mutations (~90%) are associated with the deletion mutations in exon 19 and L858R mutations in exon 21, although the EGFR mutations are distributed throughout the tyrosine kinase encoding region (17).

Previous clinical findings have shown limited success with regard to NSCLC targeting therapy, such as the those from Canada (NCIC-BR19) (18). In that study, gefitinib and placebo were used in patients with IB-IIIA stage of NSCLC, who underwent radical surgery and the results showed that the experimental group failed to obtain differential DFS and OS. A retrospective study conducted in the United States (MSKCC) reported that (19): compared to platinum-based chemotherapy, TKI-assisted therapy (gefitinib and erlotinib) had a trend of prolonging DFS for 2 years in 167 NSCLC cases with EGFR mutation (stage IB accounted for 70%, stage II accounted for 15%, and stage III accounted for 15%) (89 vs. 72%, P=0.06). The SELECT study reported at the 2012 ASCO conference, investigated the erlotinib application in the maintenance treatment following the adjuvant chemotherapy of NSCLC with EGFR mutation (20). The preliminary results showed that following maintenance therapy with erlotinb, the median of the 2-year survival rate without diseases was 94%. Only 1 case exhibited tumor progression during maintenance therapy with erlotinb, 10 cases exhibited tumor progression subsequent to drug withdrawal for 6 months, and 5 cases were sensitive to retreatment of erlotinib. Of note, patients in stage I accounted for 53% in this study with a survival curve well below the curve of stage III patients (20). The findings suggested that patients in relatively early stages of cancer had no obvious benefits in that study. Maintenance treatment with erlotinib was identified to inhibit cell growth for micrometastatic lesions at least.

The focus of the present study was on patients with advanced non-small lung cancer and malignant pleural effusion and provided targeted therapy for patients with EGFR mutations. The results of the present study showed that the 6-month survival rate, PFS, objective response rate and disease control rate of the experimental group were higher than those in the control group and the differences were statistically significant. The drug side-effects of the experimental group were not statistically different compared to the control group. However, the presence or absence of EGFR mutations revealed no correlation with gender, age, tumor type, stage and the size of the tumor. Although the present study had a small number of samples and shorter observation indices, EGFR mutations were detected for advanced (stages III-IV) lung cancer patients. Cases were provided with targeted therapy and the follow-up data showed that the 6-month survival rate was 51.2%, the average PFS was 5.7 months, RP was 56.1%, DCR was 58.5%, and that the drug side-effects were only 17.1%. These findings show that our experiments achieved acceptable results. We conclude that EGFR mutations have a higher incidence in patients with advanced non-small lung cancer and malignant pleural effusion and the targeted therapy was a safe and effective method for these patients with mutations, which may further improve their survival rate.

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