Oncology and Translational Medicine

Volume 3 • Number 3 • June 2017

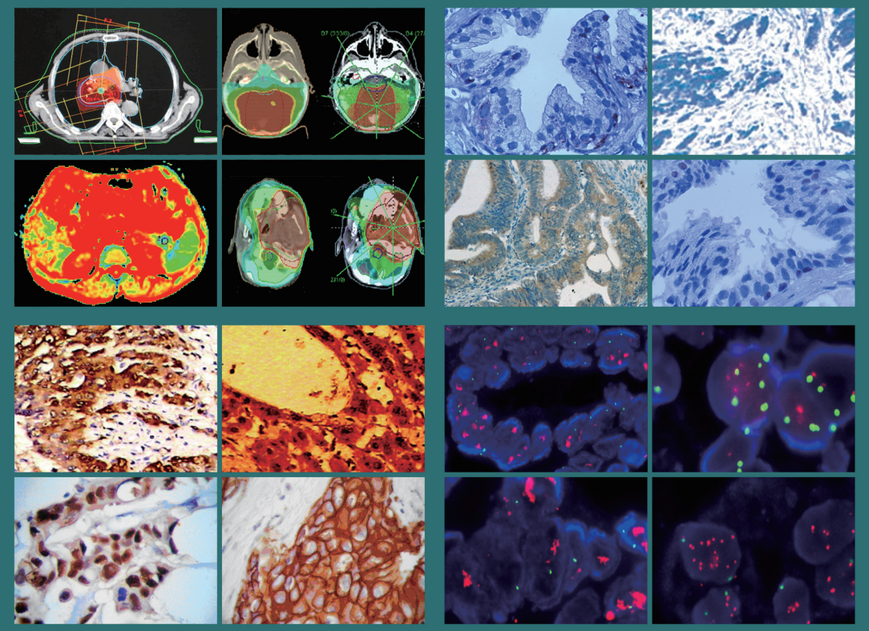
Oncology and Translational Medicine Volume 3 • Number 3 • June 2017 pp 91–135

邮发代号：38-121
ISSN 2095-9621

GENERAL INFORMATION
≫otm.tjh.com.cn

ISSN 2095-9621
CN 42-1865/R

Oncology and Translational Medicine



Volume 3
Number 3
June 2017

Oncology and Translational Medicine

Oncology and Translational Medicine
June 2017 Volume 3 Number 3

Contents

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0214-4
June 2017, Vol. 3, No. 3, P91–P92

EDITORIAL

Whole process control and precision therapy in lung cancer

Zhaozhe Liu, Xiaodong Xie (✉)

Oncology Department, General Hospital of Shenyang Military Region, Shenyang 110016, China

Xiaodong Xie Professor and Chairman, Cancer Center of People's Liberation Army, General Hospital of Shenyang Military Region, China. In 1980, he entered the Fourth Military Medical University of PLA in Xi'an, and he acquired a Master's degree in Urology in 1996. From 2000 to 2001, he studied as a research fellow in the Department of Oncology, Cancer Institute & Hospital, Chinese Academy of Medical Sciences. At present, he is a doctoral supervisor at the Liaoning University of Chinese Medicine and the Fourth Military Medical University. He is a member of the council of the Chinese Anti-Cancer Association (CACA), a member of the standing committee of the Professional Committee of Cancer Rehabilitation and Palliative Care (CRPC) of the CACA, a member of the standing committee of the Professional Committee of Tumor Markers of the CACA, an Executive Member of the Chinese Society of Clinical Oncology (CSCO), a Member of the Standing Committee of the Chinese Medical Doctor Association (CMDA), and Vice Chairman of the Cancer Professional Committee of the People's Liberation Army. In 2012, he received the Second Award of Scientific and Technological Advancement in Liaoning Province, and received the Outstanding Paper Award at the annual meeting of the CACA-CRPC.

Lung cancer is one of the most common malignant tumors in the world. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer cases, and approximately 75% of patients are diagnosed in the middle and late stages. The treatment methods mainly include surgery, chemotherapy, radiotherapy, molecular targeted therapy, traditional Chinese medicine therapy, and immune therapy. We summarize the current status of lung cancer-related treatment options and targets.

Cisplatin-based regimens are given priority for chemotherapy in NSCLC, but chemotherapy induced nausea and vomiting (CINV) is a common and debilitating side effect. The most common anti-emetic drugs are the first generation of 5-hydroxytryptamine-3 (5-HT3) serotonin receptor antagonists, which have poor efficacy. Palonosetron is a new 5-HT receptor antagonist, with a long half-life and a strong affinity that is more than 100 times that of the first-generation 5-HT receptor antagonists. In our study, the efficiency and control rate of palonosetron versus aprepitant have an obvious advantage compared with tropisetron; palonosetron combined with aprepitant has a remarkable effect on acute and delayed vomiting caused by cisplatin-based regimens.

Recently, maintenance chemotherapy has been extensively investigated for NSCLC; the purpose is to gain a maximum effect of tumor control after delivering systematic treatment. Etoposide is an important chemotherapeutic agent used for the treatment of a wide spectrum of human cancers. Thalidomide is an oral anti-angiogenic agent, which inhibits angiogenesis mediated by vascular endothelial growth factor (VEGF), basic fibroblast growth factors and microvessel formation in experimental models. If NSCLC patients have a stable response after first-line four to six cycles of platinum-based therapy, then such patients upon treatment with etoposide plus thalidomide have a significantly longer progression-free survival (PFS) with tolerable toxicity in maintenance therapy for advanced NSCLC.

Molecular targeted therapy has been the main treatment option in our clinic. For patients with *EGFR* mutations, tyrosine kinase inhibitors (TKI) can obviously prolong the patient's PFS and overall survival as compared with chemotherapy. However, disease progression occurs rapidly for patients who develop resistance to TKIs.

✉ Correspondence to: Xiaodong Xie. Email: doctor_xxd@163.com.

Hence, we observed the efficacy and safety of pemetrexed combined with continuous daily administration of erlotinib or gefitinib in advanced-stage NSCLC patients undergoing TKI treatment. The treatment showed a higher objective response rate (ORR) and disease control rate (DCR) among patients, and the adverse effects were well tolerated. Pemetrexed combined with erlotinib or gefitinib may be more efficient than the conventional second-line treatments (pemetrexed, docetaxel, and *EGFR* TKIs) in NSCLC.

TKI-based treatment combined with chemotherapy has a better clinical benefit, but the combined treatment has been extremely limited for elderly patients because of poor tolerance and adverse reactions. However, traditional Chinese medicine has been widely used in China as adjuvant treatment during chemotherapy and radiotherapy. Our study analyzed the efficacy and adverse reactions of TKI treatment combined with Kanglaite injections (KLTs) in elderly patients with NSCLC, and we found that the administration of KLT combined with erlotinib or gefitinib has a better curative effect and that this drug combination is well tolerated by the patients. KLT combined with TKI treatment might provide a satisfactory therapeutic strategy for elderly NSCLC patients.

Although surgery is the only treatment that can cure patients, many patients often have lost the chance of surgery. For patients without *EGFR* mutations, stimulating the body's immune system by immunotherapy may improve the effect of antitumor immunity, and has become a new kind of treatment for cancer. Programmed cell death-1 (PD-1) and its ligand PD-L1 can regulate the tumor microenvironment and mediate immune escape of tumor cells. Monoclonal antibodies against PD-1 and PD-L1 are proven to be safe and effective in patients with NSCLC, not only in elderly patients with poor organ function, but also in advanced stage patients with poor performance status who refuse to accept radiotherapy and chemotherapy. Immunotherapy will offer a new direction and bring new hope for the treatment of NSCLC.

At present, chemotherapy and molecular targeted therapy have been the main treatment methods of lung cancer, especially for the patients who have lost the chance of surgery. Increasing the antitumor activity and reducing the adverse effects are the keys to breakthroughs in traditional therapy. Through the development of personalized gene sequencing techniques and molecular biological mechanism research, new targets and molecular pathways will provide a new direction for the treatment of lung cancer. The treatment of these tumors is gradually achieving the transition from macro to micro, realizing the application of individual precision medicine in the future.

DOI 10.1007/s10330-016-0214-4
Cite this article as: Liu ZZ, Xie XD. Whole process control and precision therapy in lung cancer. Oncol Transl Med, 2017, 3: 91–92.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0209-9

June 2017, Vol. 3, No. 3, P93–P98

ORIGINAL ARTICLE

Efficacy of pemetrexed combined with erlotinib/gefitinib in advanced non-small cell lung cancer patients during tyrosine kinase inhibitor treatment*

Guanzhong Zhang[1], Zhaozhe Liu (Co-first author)[1], Tao Han[1], Xiaodong Xie[1] (✉), Shunchang Jiao[2] (✉)

[1] Department of Oncology, General Hospital of Shenyang Military Region, Shenyang 110016, China
[2] Department of Oncology, Chinese PLA General Hospital, Beijing 100853, China

Abstract

Objective We aimed to evaluate the efficacy and safety of pemetrexed combined with erlotinib/gefitinib in advanced non-small cell lung cancer (NSCLC) patients during tyrosine kinase inhibitor (TKI) treatment.

Methods Thirty-two patients with advanced NSCLC were divided into two groups. Patients in the control group received continuous daily epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) treatment, and patients in the experimental group received continuous daily EGFR-TKI along with pemetrexed treatment, which was administered on day 1 at 500 mg/m^2. Erlotinib (150 mg) or gefitinib (250 mg) was administered daily from day 1 to day 21, with a cycle of every 21 days. Dexamethasone, folic acid, and vitamin B_{12} were also administered during the treatment. The endpoint of the primary study was the disease control rate.

Results The objective response rate was 21.9% (95% CI: 7.6% to 36.3%) in the control group, whereas the disease control rate was 84.4% (95% CI: 71.8% to 97.0%) in the experimental group. The median progression-free survival was 6.2 (95% CI: 2.4 to 10.0). Grades 3 or 4 adverse effects of leucopenia (15.6%), neutropenia (12.5%), anemia (3.1%), and nausea or vomiting (3.1%) were found in the experimental group.

Conclusion The administration of pemetrexed combined with erlotinib or gefitinib showed a higher efficacy in TKI-resistant NSCLC patients. Further, the adverse effects of this drug combination were well tolerated by the patients. Pemetrexed combined with TKI treatment might provide a satisfactory therapeutic strategy for advanced NSCLC patients after TKI treatment.

Key words: non-small cell lung cancer; pemetrexed; erlotinib; gefitinib; resistance

Received: 28 November 2016
Revised: 6 February 2017
Accepted: 12 April 2017

Lung cancer is the most common cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancer cases. At diagnosis, 65% to 75% of NSCLC are locally advanced or metastatic cases and are unresectable [1–3]. Combination chemotherapy is the standard treatment for advanced NSCLC. Platinum-based chemotherapy with pemetrexed or a third-generation agent, such as gemcitabine, docetaxel, paclitaxel, or vinorelbine, has significantly improved the median survival and quality of life in patients with advanced NSCLC. For epidermal growth factor receptor (EGFR)-mutation patients, treatment with EGFR tyrosine kinase inhibitors (TKIs), namely, erlotinib or gefitinib, results in a longer progression-free survival (PFS) and better overall survival (OS) than with chemotherapy [4–6]. Maintenance treatment with TKIs after chemotherapy can also prolong the PFS and OS of patients with unknown EGFR status [7]. However, disease progression is rapid for patients who develop resistance to TKIs, and no standard treatment option is available for TKI-resistant patients [8].

Two preclinical studies showed that combined administration of pemetrexed and erlotinib had synergistic effects on human NSCLC cells [9–10]. Our preclinical study

✉ Correspondence to: Xiaodong Xie. Email: doctor_xxd@163.com; Shunchang Jiao. Email: crzs281@tom.com
* Supported by a grant from the Postdoctoral Science Foundation of China (No. 2012M512119).

also showed that combined administration of pemetrexed and gefitinib had synergistic effects on human colorectal cancer cells, and such effects were significant in gefitinib-resistant cells [11]. TKI-treated patients showing progressive disease are resistant to TKI. Therefore, pemetrexed combined with erlotinib or gefitinib may exhibit satisfactory efficacy in NSCLC patients who undergo TKI treatment. In this study, we collected patients' clinical data to evaluate the efficacy and safety of pemetrexed combined with erlotinib or gefitinib in advanced NSCLC patients.

Materials and methods

Patients

A total of 32 advanced NSCLC patients were divided into two groups in the study, which was conducted from June 2013 to March 2016; the patients met the following criteria: results of consecutive histological and pathological tests indicated disease progression during TKI treatment. Other inclusion criteria included age ≤ 75 years; life expectancy of greater than 8 weeks; Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1; adequate hematologic values (absolute neutrophil count ≥ 1.5×10^9/L, platelet count ≥ 100×10^9/L, and hemoglobin concentration ≥ 9 g/dL); normal hepatic function (total bilirubin concentration was less than 1-fold of the upper limit of the normal value, and alanine aminotransferase concentration was less than 1.5-fold of the upper limit of the normal value or elevated up to 3-fold of the upper limit of the normal value in patients with known hepatic metastases); and normal renal function (calculated creatinine clearance rate of > 45 mL/min).

Study medication

Patients in the control group received continuous daily EGFR-TKI treatment, and patients in the experimental group received continuous daily EGFR-TKI along with pemetrexed treatment. Pemetrexed at 500 mg/m^2 was intravenously administered for 10 min on day 1, then erlotinib (150 mg) or gefitinib (250 mg) was orally administered from day 1 to day 21, and the cycle was repeated every 21 days. Erlotinib/gefitinib was administered even after patients exhibited disease progression during erlotinib treatment. Folic acid at 400 μg per day was orally administered starting from 1 week prior to the first dose of pemetrexed to 3 weeks after the end of therapy. Vitamin B12 (1,000 μg) was intramuscularly injected a week before day 1 of cycle 1, and the injection was repeated every 9 weeks until the end of the study. Dexamethasone (4 mg) was orally administered twice daily on the day before and after each dose of pemetrexed. If intolerable adverse effects occurred, treatments were delayed for up to 42 d to allow the patient to recover from toxicity. Therapy was resumed using only 75% of the previous dose when the adversely affected patients exhibited 3 or 4 grade on the Common Toxicity Criteria (CTC). A patient was excluded from the study if he or she required more than 42 d of recovery time or more than two reductions in dose because of toxicity. If radiotherapy was required by the patient, treatment was discontinued until 2 weeks after the completion of radiotherapy.

Assessments and statistical methods

Baseline tumor measurements were obtained at or less than 1 week before the start of treatment. Measurements were performed using the Response Evaluation Criteria in Solid Tumors version 1.1; tumor response was assessed using the same imaging technique that was used to obtain the baseline measurements [12]. The best response was recorded at the end of the treatment period. Safety measures, such as adverse effect monitoring, physical examinations, and clinical laboratory tests (hematology, blood biochemistry, and hepatic and renal function) were performed weekly. Toxicity was graded using version 2.0 of the National Cancer Institute CTC.

Statistical analysis was performed using the Statistical Package for Social Science v. 17.0. A statistical summary of patient characteristics and efficiency and safety variables was obtained [13]. Frequencies were reported as numbers and percentages. The objective response to chemotherapy was defined by the overall best response during treatment. PFS time was defined as the duration from enrollment in the study to disease progression or death. The OS time was defined as the duration from enrollment in the study to the time of death from any cause. PFS and OS times were analyzed using the Kaplan-Meier method.

Ethics statement

All patients were from China and received treatment at the PLA General Hospital. All clinical investigations were approved by the PLA General Hospital Ethical Committee. All patients submitted signed consent forms prior to treatment.

Results

Between June 2013 and March 2016, a total of 32 patients (16 men and 16 women) participated in the study. The baseline patient characteristics are listed in Table 1. The median age of the patients was 56 years (range: 30 to 75 years). All patients had good performance status, with 24 patients having an ECOG performance status of 0, whereas 8 patients had an ECOG performance status of 1. Four patients had stage IIIB tumors and 28 patients had stage IV tumors. Thirty-one patients had

Table 1 Characteristics of patients (n = 32)

Characteristics of patients	n (%)
Age (years, range)	56 (30–75)
Sex	
Male	16 (50.0)
Female	16 (50.0)
Weight (kg, mean ± s)	63 ± 8.9
Stage	
IIIB	4 (12.5)
IV	28 (87.5)
ECOG Performance status	
0	24 (75.0)
1	8 (25.0)

adenocarcinoma, and 1 patient had large cell carcinoma. Twenty-six patients had mutated *EGFR* and 6 patients had unknown *EGFR* status, i.e., the *EGFR* gene status was not detected before TKI treatment because of personal reasons.

Fifteen patients received erlotinib treatment, and 17 patients received gefitinib treatment. Seven patients were treated with TKI as first-line treatment, 13 patients were treated with TKI as second- or third-line treatment, and 12 patients were given TKI as maintenance treatment after first-line chemotherapy. Among 32 patients receiving EGFR-TKI treatment, 5 patients achieved a partial response (PR) and 24 had stable disease (SD) with an objective response rate (ORR) of 15.6% and a disease control rate (DCR) of 90.6%. The median PFS was 10.2 months (95% CI: 1.1 to 16.9). All patients who showed disease progression during erlotinib or gefitinib treatment were administered pemetrexed along with daily EGFR-TKI treatment.

Two patients completed only one cycle of treatment because of a decline in general physical condition, whereas the other 30 patients received at least two cycles of pemetrexed in addition to TKI treatment. The total number of treatment cycles was 186, and the median number of treatment cycles was six (in the range of 1 to 24). Three patients (9.4%) underwent dose modification, and treatment was delayed for 8 patients because of adverse effects. No mortality was recorded among the patients at the end of the follow-up period in August 2013. All patients were evaluated for treatment efficacy and adverse reaction. At follow-up, no disease progression was observed in 13 patients, 6 patients were still in treatment, and 30 patients were still alive.

Efficacy

We failed to observe a complete response (CR) in all patients treated with pemetrexed plus erlotinib or gefitinib. Seven patients (21.9%) achieved a PR, 20 patients (62.5%) achieved SD, and 5 patients (15.6%) showed progressive disease. The ORR [(CR + PR)/n] was 21.9% (in 7 of 32 patients, 95% CI: 7.6% to 36.3%). The DCR [(CR + PR + SD)/n] was 84.4% (in 27 of 32 patients, 95% CI: 71.8% to 97.0%). The tumor response is summarized in Table 2. The median PFS was 6.2 months (95% CI: 2.4 to 10.0). The Kaplan-Meier plot for PFS is displayed in Fig. 1. The 6-month and 1-year PFS rates were 53.3% and 32.1%, respectively.

Table 2 Response for patients with NSCLC treated with pemetrexed plus erlotinib or gefitinib (n = 32)

Response	n (%)
CR	0 (0.0)
PR	7 (21.9)
SD	20 (62.5)
PD	5 (15.6)
ORR	7 (21.9)
DCR	27 (84.4)

Safety

Toxicity was evaluated in all patients and in all cycles. Twenty-one patients (65.6%) reported at least one adverse effect during the study. Five and two patients (15.6% and 6.3%, respectively) experienced grades 3 and 4 adverse effects, respectively. The common adverse effects were leucopenia (in 21 of 32 patients, 65.6%), neutropenia (in 19 of 32 patients, 59.4%), nausea or vomiting (in 18 of 32 patients, 56.3%), rash (in 17 of 32 patients, 53.1%), and diarrhea (in 10 of 32 patients, 31.3%). Grades 3 or 4 adverse effects included leucopenia (in 5 of 32 patients, 15.6%), neutropenia (in 4 of 32 patients, 12.5%), anemia (in 1 of 32 patients, 3.1%), and nausea/vomiting (in 1 of 32 patients, 3.1%). By the end of the study, 2 patients had died because of disease progression. No mortality cases were caused by treatment.

Discussion

Several randomized phase III clinical trials showed that gemcitabine or paclitaxel plus platinum combined with continuous daily administration of erlotinib or gefitinib as first-line therapy failed to improve the survival of patients with advanced NSCLC [14–17]. Thus, chemotherapy combined with EGFR-TKI was not a satisfactory approach for treating NSCLC, and few researchers were interested in using this strategy. However, another randomized phase III study showed that PFS and OS were longer in advanced pancreatic cancer patients who received continuous daily erlotinib treatment in addition to gemcitabine compared with patients who received gemcitabine alone [18].

In the current study, we observed the efficacy and safety of pemetrexed combined with continuous daily administration of erlotinib or gefitinib (from day 1 to 21) in advanced NSCLC patients undergoing TKI treatment.

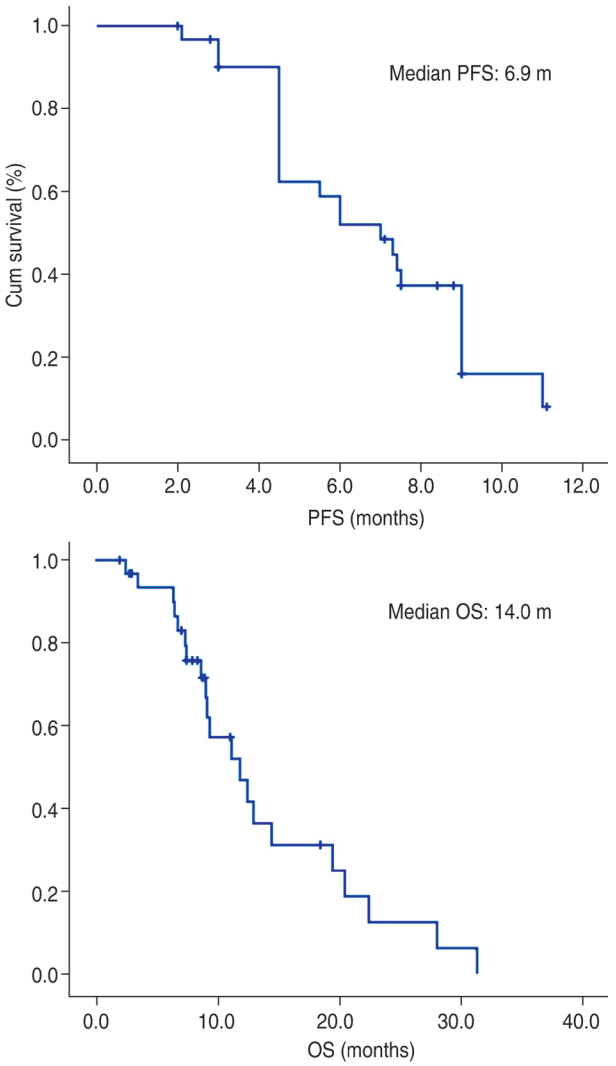


Fig. 1 PFS and OS (n = 32)

The treatment modality was effective and safe. Among 32 patients, 7 patients achieved a PR and 20 patients achieved SD. The DCR and ORR were 84.4% and 21.9%, respectively. The median PFS was 6.2 months. The grade 3 or 4 adverse effects were as follows: leukopenia, 15.6%; neutropenia, 12.5%; anemia, 3.1%; and nausea or vomiting, 3.1%.

Naruo *et al* conducted a phase II clinical study involving the administration of pemetrexed combined intermittently with gefitinib or erlotinib (from day 2 to day 16) after relapse to gefitinib or erlotinib treatment in advanced NSCLC patients [19]. In the study of Naruo *et al*, the DCR and ORR were 77.8% and 25.9%, respectively, and the median PFS was 7 months. The adverse effects were also well tolerated by the patients.

In a multicenter, international, and randomized phase III trial reported by Hanna *et al*, the effects of single-agent pemetrexed were compared with those of docetaxel for previously treated NSCLC patients. The ORR for pemetrexed and docetaxel were 9.1% and 8.8%, respectively. The DCR was 45.8% for pemetrexed and 46.8% for docetaxel [20]. Results of an international, randomized clinical trial showed that erlotinib had a similar efficacy, but lower toxicity, compared with docetaxel in 731 EGFR-unselected patients [21–23].

The above-mentioned studies determined whether pemetrexed and erlotinib could become standard second-line treatments for NSCLC. Compared with the current standard second-line treatment of NSCLC, our findings and those of Naruo *et al* showed higher ORR and DCR, and the adverse effects were well tolerated. Thus, pemetrexed combined with erlotinib or gefitinib may be more effective than the conventional second-line treatments (pemetrexed, docetaxel, and EGFR TKIs) for NSCLC.

Some preclinical studies discussed the mechanism underlying the synergistic effects of pemetrexed and TKIs. TKIs affect the expression and activity of thymidylate synthase enzyme in tumor cells, and such enzymes increase the effectiveness of pemetrexed against tumor cells [10–11]. Pemetrexed increases the activity of the EGFR signal transduction pathway, which in turn increases the independence of the cell from the pathway. The magnitude of the increase in activity is significantly reduced by TKIs [9, 11]. Previous research results explained the mechanism underlying the efficacy of pemetrexed in combination with TKI in NSCLC patients.

Li *et al* reported an antagonistic interaction between pemetrexed and erlotinib in NSCLC cells when erlotinib administration preceded that of pemetrexed. Erlotinib arrested the cell cycle at the G_1 phase and consequently reduced the effect of pemetrexed on tumor cells. In contrast, a synergistic or additive interaction was observed when pemetrexed administration preceded that of erlotinib or when pemetrexed and erlotinib were simultaneously used [9]. Naruo *et al* administered pemetrexed to patients on day 1 and TKIs on days 2 to 16 within a 21-day cycle, i.e., pemetrexed was combined with TKIs intermittently. Li *et al* suggested that the antagonistic interaction was much less in TKI-resistant cells than in TKI-sensitive cells [9]. NSCLC patients who show disease progression during TKI treatment are resistant to TKI. Thus, the antagonistic effect is expected to be low when pemetrexed is administered in combination with continuous TKI treatment.

Continuous TKI treatment may show strong synergistic effects with pemetrexed because of the long duration of tumor exposure to the drug. Moore *et al* showed that gemcitabine combined with continued daily oral erlotinib treatment showed satisfactory effects for advanced pancreatic cancer patients [18]. In Moore's study, 90% of the patients had KRAS gene mutations and

showed TKI resistance [23–24]. The same TKI treatment modality used in Moore's research was applied in the current study. No significant difference was found between the efficacy of our continuous TKI treatment and Naruo *et al*'s intermittent TKI treatment, possibly because the sample sizes were small in both trials. Further studies are required to determine whether pemetrexed combined with continuous TKI treatment can have better efficacy than pemetrexed combined with intermittent TKI treatment.

In this study, all patients who underwent TKI treatment showed progressive disease, thereby indicating their resistance to TKIs. The synergistic effect was stronger in resistant cells than in sensitive cells, consistent with our preclinical study [11]. Thus, NSCLC patients who showed progressive disease during TKI treatment responded well to the administration of pemetrexed combined with TKIs.

In conclusion, compared with conventional treatment, pemetrexed combined with continuous TKI treatment showed high efficacy and the adverse effects were well tolerated by patients with advanced NSCLC. We believe that pemetrexed combined with TKI treatment might provide a satisfactory therapeutic strategy for advanced NSCLC patients after TKI treatment. However, further studies are required to validate the effects of this combined therapeutic strategy.

Acknowledgements

We are grateful to medical personnel of Department of Oncology Medicine of Chinese PLA General Hospital for their grateful help.

References

1. Ellison G, Zhu G, Moulis A, *et al*. EGFR mutation testing in lung cancer: a review of available methods and their use for analysis of tumour tissue and cytology samples. J Clin Pathol, 2013, 66: 79–89.
2. Saintigny P, Burger JA. Recent advances in non-small cell lung cancer biology and clinical management. Discov Med, 2012, 13: 287–297.
3. Zhang GZ, Jiao SC, Meng ZT. Pemetrexed plus cisplatin/carboplatin in previously treated locally advanced or metastatic non-small cell lung cancer patients. J Exp Clin Cancer Res, 2010, 29: 38.
4. Maemondo M, Inoue A, Kobayashi K, *et al*. North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med, 2010, 362: 2380–2388.
5. Zhou C, Wu YL, Chen G, *et al*. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small- cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol, 2011, 12: 735–742.
6. Rosell R, Carcereny E, Gervais R, *et al*. Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol, 2012, 13: 239–246.
7. Chen X, Liu Y, Røe OD, *et al*. Gefitinib or erlotinib as maintenance therapy in patients with advanced stage non-small cell lung cancer: a systematic review. PLoS One, 2013, 8: e59314.
8. Oxnard GR, Arcila ME, Chmielecki J, *et al*. New strategies in overcoming acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer. Clin Cancer Res, 2011, 17: 5530–5537.
9. Li T, Ling YH, Goldman ID, *et al*. Schedule-dependent cytotoxic synergism of pemetrexed and erlotinib in human non-small cell lung cancer cells. Clin Cancer Res, 2007, 13: 3413–3422.
10. Giovannetti E, Lemos C, Tekle C, *et al*. Molecular mechanisms underlying the synergistic interaction of erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, with the multitargeted antifolate pemetrexed in non-small-cell lung cancer cells. Mol Pharmacol, 2008, 73: 1290–1300.
11. Zhang G, Xie X, Liu T, *et al*. Effects of pemetrexed, gefitinib, and their combination on human colorectal cancer cells. Cancer Chemother Pharmacol, 2013, 72: 767–775.
12. Therasse P, Arbuck SG, Eisenhauer EA, *et al*. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst, 2000, 92: 205–216.
13. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. Biometrics, 1982, 38: 143–151.
14. Herbst RS, Prager D, Hermann R, *et al*. TRIBUTE: A phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapyi n advanced non-small-cell lung cancer. J Clin Oncol, 2005, 23: 5892–5899.
15. Giaccone G, Herbst RS, Manegold C, *et al*. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trialK INTACT1. J Clin Oncol, 2004, 22: 777–784.
16. Herbst RS, Giaccone G, SchillerJH, *et al*. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trialK INTACT 2. J Clin Oncol, 2004, 22: 785–794.
17. Herbst RS, Prager D, Hermann R, *et al*. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol, 2005, 23: 5892–5899.
18. Moore MJ, GoldsteinD, HammJ, *et al*. Erlotinib plus gemcitabine compared to gemcitabine alone in patients with advanced pancreatic cancer. A phase III trial of the National Cancer Institute of Canada Clinical Trials Group [NCIC-CTG]. Am Soc Clin Oncol Annu Meet, 2005, 23: 1.
19. Yoshimura N, Okishio K, Mitsuoka S, *et al*. Prospective assessment of continuation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of pemetrexed. J Thorac Oncol, 2013, 8: 96–101.
20. Hanna N, Shepherd FA, Fossella FV, *et al*. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncologist, 2004, 22: 1589–1597.
21. Leighl NB. Treatment paradigms for patients with metastatic non-small-cell lung cancer: first-, second-, and third-line. Curr Oncol, 2012, 19(Suppl 1): S52–58.
22. Kim ES, Hirsh V, Mok T, *et al*. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (interest): a randomized phase III trial. Lancet, 2008, 372: 1809–1818.
23. Shepherd FA, Pereira JR, Ciuleanu T, *et al*. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st and 2nd line chemotherapy. A

National Cancer Institute of Canada Clincal Trials Group (NCIC CTG) Trial. Proc Am Soc Clin Oncol, 2004, (Abstract #7002).
24. Li T, Lara PN Jr, Mack PC, *et al*. Intercalation of erlotinib and pemetrexed in the treatment of non-small cell lung cancer. Curr Drug Targets, 2010, 11: 85–94.

DOI 10.1007/s10330-016-0209-9
Cite this article as: Zhang GZ, Liu ZZ, Han T, *et al*. Efficacy of pemetrexed combined with erlotinib/gefitinib in advanced non-small cell lung cancer patients during tyrosine kinase inhibitor treatment. Oncol Transl Med, 2017, 3: 93–98.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0208-8
June 2017, Vol. 3, No. 3, P99–P102

ORIGINAL ARTICLE

The efficacy of Kanglaite injection during treatment with tyrosine kinase inhibitor in elderly patients with non-small cell lung cancer*

Wei Zhou[1], Tao Han (Co-first author)[2], Zhaozhe Liu[2], Xiaodan Yang[2], Yu Liu[1], Wei Wang[1], Benqiang Yang [1] (✉), Xiaodong Xie[2] (✉)

[1] Department of Diagnostic radiology, The General Hospital of Shenyang Military Region, Shenyang 110016, China
[2] Department of Oncology, General Hospital of Shenyang Military Region, Shenyang 110016, China

Abstract

Objective Epidermal growth factor receptor–tyrosine kinase inhibitors (EGFR–TKIs) are widely used in the treatment of EGFR mutation-positive non-small cell lung cancer (NSCLC) patients. The Kanglaite injection (KLT) is a novel broad-spectrum anti-cancer injection produced from traditional Chinese medicinal herbs (coix seed). After its approval in 1995, KLT has become the most popular anti-cancer drug in China. As of this writing, no standard treatment guideline is available for elder patients with NSCLC, and the role of traditional Chinese medicinal herbs, including KLT, combined with TKI treatment remains unknown. This retrospective study evaluated the efficacy and safety of KLT in elderly NSCLC patients during TKI treatment.

Methods Thirty elderly patients aged 71-79 years with histopathologically confirmed NSCLC attending the General Hospital of the Shenyang Military Region were enrolled in the study and received EGFR-TKI treatment. All participants received 200 mL KLT injections at the same time on days 1–21. Erlotinib (150 mg) or gefitinib (250 mg) was administered daily from days 1 to 21, and the cycle was repeated every 21 days. The endpoint of the primary study was the disease control rate.

Results Thirty elderly patients were enrolled in this study. The objective response rate was 21.3% [95% confidence interval (CI): 8.6% to 35.2%], whereas the disease control rate was 80.4% (95% CI: 71.8% to 97.0%). The grade 3 or 4 adverse effects included leucopenia (13.7%), neutropenia (13.4%), anemia (2.9%), and nausea or vomiting (2.7%).

Conclusion The administration of KLT combined with erlotinib or gefitinib showed high efficacy in elderly NSCLC patients. The adverse effects of the drug combination were well tolerated by the patients. KLT combined with TKI treatment might provide a satisfactory therapeutic strategy for elderly NSCLC patients.

Received: 4 August 2016
Revised: 4 September 2016
Accepted: 25 September 2016

Key words: non-small cell lung cancer (NSCLC); Kanglaite injection (KLT); epidermal growth factor receptor–tyrosine kinase inhibitor (EGFR-TKI)

Lung cancer is characterized by malignant tumors and has the highest morbidity and mortality rates worldwide. About 1.60 million new patients are diagnosed with lung cancer, and 1.38 million patients die of lung cancer each year. Non-small cell lung cancer (NSCLC) is a lung cancer with poor prognosis that is resistant to chemotherapy [1]. Molecular targeted therapy has been the main clinical treatment option; in epidermal growth factor receptor (EGFR) mutation-positive patients, EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib or gefitinib have shown a longer progression-free survival (PFS) and overall survival (OS) in NSCLC patients [2]. Recent studies have shown that TKI-based treatment combined with chemotherapy offers better clinical benefits. However, the used of combined treatment has been extremely limited in elderly patients due to poor tolerance and adverse reactions. Thus, identification of good therapeutic options that allow elderly patients to benefit from TKI-based treatment is of great clinical significance.

In recent years, traditional Chinese medicine has been

✉ Correspondence to: Benqiang Yang. E-mail: crzs281@tom.com; Xiaodong Xie. Email: doctor_xxd@163.com
* Supported by a grant from the China Postdoctoral Science Foundation Grant (No. 2015M582822).

widely used in China as an adjuvant treatment during chemotherapy and radiotherapy for cancer, offering good therapeutic effects [3]. Kanglaite injection (KLT) is a Chinese herbal compound that has been widely used for the treatment of non-small-cell lung, liver, and gastric cancer in China [4]. KLT has been administered to approximately 6 million patients with these cancers. The main ingredient of KLT is seed oil, which has been widely used for cancer treatment and has good therapeutic effects on cancer metastasis and immunological disorders [5]. Preclinical studies have found that KLT may block the tumor cell cycle at the G2/M phase and induce tumor cell apoptosis by up-regulating the expression of Fas/Apo-1 and down-regulating the expression of Bcl-2 and COX-2 [6–7]. KLT significantly decreases cancer cachexia, improves the quality of life of cancer patients, and may ameliorate multiple drug resistance in cancers when combined with radiotherapy and chemotherapy in clinical use.

Our study analyzed the effects and adverse reactions of TKI treatment combined with KLT injection in elderly patients with NSCLC. The results suggest that this combined treatment might provide a satisfactory therapeutic strategy for elderly NSCLC patients.

Materials and methods

Patients

Thirty-two elderly patients (71–79 years of age) with histopathologically confirmed NSCLC attending the General Hospital of Shenyang Military Region were enrolled in the study. All participants received continuous daily EGFR-TKI treatment and simultaneous KLT injections. Before treatment, these patients had Eastern Cooperative Oncology Group (ECOG) performance scores between 0–1 and no obvious abnormal blood, liver and kidney function, and electrocardiogram findings.

Treatment

All patients were administered oral erlotinib (150 mg) or gefitinib (250 mg) daily from days 1 to 21, and the cycle was repeated every 21 days. The KLT injection (200 mL) was also administered daily on days 1 to 21. The endpoint of the primary study was the disease control rate. If any patient could not tolerate the adverse reactions during treatment, the dosage was reduced by 20% in the next treatment cycle. The treatments were stopped if the patients still could not tolerate the adverse reactions. After two cycles of TKI combined with KLT treatment, the therapeutic effects were evaluated. The treatments were terminated if disease progression or intolerable adverse reactions occurred.

Assessments and statistical methods

Baseline tumor measurements were taken no more than one week before the start of treatment. According to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, tumor response was evaluated using the same imaging technique that was used at baseline. At the end of the treatment period, the best tumor response was recorded. Safety measures including adverse events, physical examinations, and clinical laboratory tests (hematology, blood biochemistry, hepatic functions, and renal functions) were assessed weekly. Toxicity was graded using version 2.0 of the National Cancer Institute Common Toxicity Criteria.

Statistical analysis was performed using PASW Statistics for Windows, Version 18.0. Differences among variables were assessed by two-tailed Student's *t*-tests. Data were presented as the means ± standard error of the mean unless otherwise indicated. $P<0.05$ was considered statistically significant.

Ethics statement

All patients were from China and received treatment in our hospital. All clinical investigations were approved by the General Hospital of Shenyang Military Region Ethical Committee and all patients involved in this study signed consent forms.

Results

Between June 2013 and March 2016, a total of 30 patients were enrolled in the study. The baseline patient characteristics were listed in Table 1. The median age was 75 years (range, 71–79) and there were 16 male and 14 female patients. All patients had a good performance status; 24 and 8 had ECOG performance status scores of 0 and 1, respectively. Sixteen patients had stage IIB tumors, while 14 patients had stage IV tumors. Twenty-nine patients had adenocarcinoma and one patient had large cell carcinoma. Among the 30 patients, 19 were treated with TKI in the first round, and 11 were treated in second or third rounds of treatment. Five patients achieved partial response (PR) and 21 had stable disease (SD); the objective response rate (ORR) was 16.7% and the disease control rate (DCR) was 86.7% in the 30 patients who received the combined EGFR-TKI and KLT treatment. All patients received at least two cycles of KLT plus TKI. At the end of the follow-up in March 2016, no patients were lost to follow-up, and the efficacy and adverse reaction were evaluated for patients.

In brief, of the 30 patients treated with KLT plus erlotinib/gefitinib, no complete response (CR) was observed; five patients (5/30, 16.7%) achieved PR, 21 patients (21/30, 70%) achieved SD, and four patients (4/30, 13.3%) had progressive disease (PD). The ORR [(CR+PR)/*n*] was 16.7% (5/30, 95% CI: 6.5-37.8%) and

Table 1 Patient characteristics (Total=30)

Characteristics of patients	*n*	%
Age (years, range)	75	71–79
Sex		
Male	16	53.3
Female	14	46.7
S (m^2, mean ± s)	1.83 ± 0.18	
Stage		
IIB	16	53.3
IV	14	46.7
ECOG Performance status		
0	22	73.3
1	8	26.7

Table 2 Response in elderly patients with NSCLC treated with EGFR-TKIs plus KLT (Total=30)

Response	*n*	%
CR	0	0
PR	5	16.7
SD	21	70.0
PD	4	13.3
ORR	16.7	
DCR	86.7	

Table 3 Toxicity in patients (*n*, %)

Toxicity	I/II	III/IV
Nausea/vomiting	2 (6.7)	1 (3.3)
ALT/AST	6 (20)	2 (6.7)
Rash	24 (80)	1 (3.3)
Pyrexia	2 (6.7)	0 (0)
Fatigue	9 (30)	0 (0)

the DCR [(CR+PR+SD)/n] was 86.7% (26/30, 95% CI: 70.2–97.0%). The tumor responses were summarized in Table 2.

Discussion

Several randomized phase III clinical trials of gemcitabine or paclitaxel plus platinum in combination with continuous daily administration of erlotinib or gefitinib as first-line therapy failed to show improved survival in patients with advanced NSCLC [8]. This led to the conclusion that chemotherapy combined with EGFR-TKI is not a good choice and there has been little interest in pursuing such strategies. However, a later randomized phase III study showed significant improvements in progression-free and overall survival in patients with advanced pancreatic cancer receiving continuous daily erlotinib in addition to gemcitabine compared with gemcitabine alone [9]. The current study assessed the efficacy and safety of KLT combined with the continuous daily administration of erlotinib or gefitinib in progressive NSCLC patients during TKI-based treatment. The results showed that the treatment modality was effective and safe in those patients. Out of 30 patients, five and 21 achieved PR and SD, respectively. The grade 3 and 4 adverse events included nausea/vomiting (3.3%), abnormal liver function(6.7%), and rash (3.3%). And the toxicity of patients were summarized in Table 3. In that study, the DCR and ORR were 77.8% and 25.9%, respectively. The adverse events were also well tolerated by patients. These results showed that compared with TKI treatment for NSCLC, the patients in the current study showed better ORR and DCR and the adverse events were well tolerated with combined treatment; moreover, KLT combined with erlotinib/gefitinib may offer better efficiency than EGFR-TKI for the treatment of NSCLC.

Preclinical studies have shown that TKI influences the expression and activity of thymidylate synthase in tumor cells, which increases the killing effect of pemetrexed [10]. Studies also found that KLT may block the tumor cell cycle at the G2/M phase and induce tumor cell apoptosis by up-regulating the expression of Fas/Apo-1 and down-regulating the expression of Bcl-2 and COX-2. KLT also has been found to significantly decrease cancer cachexy and improve the quality of life of cancer patients, which might enhance the TKIs efficiency. However, more basic research is needed to explain the mechanism by which KLT in combination with TKI offers better efficacy in NSCLC patients.

In conclusion, our study showed that KLT combined with continuous TKI treatment offered better efficacy and tolerability in patients with progressive NSCLC patient during TKIs treatment. The study suggested that KLT combined with TKI treatment might be a good strategy in progressive NSCLC patients.

Competing interests

We declare no conflicts of interests.

Acknowledgements

The authors would like to thank Fang Guo for his technical assistance.

References

1. Carrera S, Buque A, Aresti E, *et al*. Epidermal growth factor receptor tyrosine-kinase inhibitor treatment resistance in non-small cell lung cancer: biological basis and therapeutic strategies. Clin Transl Oncol, 2014,164: 339–350.
2. Kumarakulasinghe NB, van Zanwijk N, Soo RA. Molecular targeted therapy in the treatment of advanced stage non-small cell lung cancer (NSCLC). Respirology, 2015, 203: 370–378.
3. Ma LX,P. Ai P, Li H, *et al*. The prophylactic use of Chinese herbal

medicine for chemotherapy-induced leucopenia in oncology patients: a systematic review and meta-analysis of randomized clinical trials. Support Care Cancer, 2015, 23: 561–579.
4. Pan P, Wu Y, Guo ZY, *et al*. Antitumor activity and immunomodulatory effects of the intraperitoneal administration of Kanglaite in vivo in Lewis lung carcinoma. J Ethnopharmacol, 2012,1432: 680–685.
5. Zhan YP, Huang XE, Cao J, *et al*. Clinical safety and efficacy of kanglaite® (coix seed oil) injection combined with chemotherapy in treating patients with gastric cancer. Asian Pac J Cancer Prev, 2012,13: 5319–5321.
6. Lu Y, Li CS, Dong Q. Chinese herb related molecules of cancer-cell-apoptosis: a minireview of progress between Kanglaite injection and related genes. J Exp Clin Cancer Res (Online), 2008, 27: 31.
7. Lv P, Zhou K, Zheng Z, *et al*. Effect of Kanglaite injection on cell apoptosis of human pneumonic adenocarcinoma cell line A 549 and its mechanism. Modern J Integrated Trad Chin Western Med, 2010, 25: 3156–3158.
8. Casal Rubio J, Fírvida-Pérez JL, Lázaro-Quintela M, *et al*. A phase II trial of erlotinib as maintenance treatment after concurrent chemoradiotherapy in stage III non-small-cell lung cancer (NSCLC): a Galician Lung Cancer Group (GGCP) study. Cancer Chemother Pharmacol, 2014, 733: 451–457.
9. Yoshioka H, Azuma K, Yamamoto N, *et al*. A randomized, double-blind, placebo-controlled, phase III trial of erlotinib with or without a c-Met inhibitor tivantinib (ARQ 197) in Asian patients with previously treated stage IIIB/IV nonsquamous nonsmall-cell lung cancer harboring wild-type epidermal growth factor receptor (ATTENTION study). Ann Oncol, 2015, 26: 2066–2072.
10. Ping-Chih Hsu,Chien-Ying Liu,Shih-Hong Li,Shih-How Huang,Chih-Liang Wang,Chih-Hsi Kuo,Fu-Tsai Chung,Chih-Hung Chen,Chih-Teng Yu,Cheng-Ta Yang. Efficacy of platinum-based combination chemotherapy in advanced lung adenocarcinoma harboring sensitive epidermal growth factor receptor (EGFR) mutations with acquired resistance to first-line EGFR tyrosine kinase inhibitor (TKI). Cancer Treatment and Research Communications, 2016, 9: 48–55.

DOI 10.1007/s10330-016-0208-8
Cite this article as: Zhou W, Han T, Liu ZZ, *et al*. The efficacy of Kanglaite injection during treatment with tyrosine kinase inhibitor in elderly patients with non-small cell lung cancer. Oncol Transl Med, 2017, 3: 99–102.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0207-7

June 2017, Vol. 3, No. 3, P103–P107

ORIGINAL ARTICLE

Effect of etoposide plus thalidomide as maintenance therapy on progression-free survival of elderly patients with advanced non-small cell lung cancer

Yanan Ge, Zhendong Zheng (Co-first author), Zhaozhe Liu, Jianing Qiu, Xiaodong Xie (✉)

Department of Oncology, General Hospital of Shenyang Military Region, Shenyang 110016, China

Abstract

Objective The aim of the study was to evaluate the efficacy and safety of etoposide plus thalidomide as maintenance therapy for elderly patients with advanced non-small cell lung cancer (NSCLC) without disease progression after first-line chemotherapy.

Methods After four to six cycles of platinum-based first-line therapy, 64 elderly patients with advanced NSCLC without disease progression who were treated in the General Hospital of Shenyang Military Region (China) from 2014 to 2016 were enrolled in this study. According to the different maintenance treatment methods, patients were divided as having received etoposide plus thalidomide therapy (treatment group, n = 32) and best supportive care (control group, n = 32). Disease control and progression-free survival (PFS) were compared between the two groups.

Results The recent curative effect objective response rates of the treatment group and the control group were 31.3% and 3.1%, respectively, and the disease control rates were 71.9% and 31.3%, respectively. The Kaplan-Meier survival curves of the two groups were significantly different (χ^2 = 26.532, P = 0.001). The median PFS for the treatment group and control group was 6.0 months [95% confidence interval (CI) = 4.3–7.9 months] and 3.2 months (95% CI = 2.6–3.8 months), respectively. The side effects in the treatment group included hematologic abnormalities, gastrointestinal toxicity, and impaired liver function, which were relieved after symptomatic support therapy and drug withdrawal.

Conclusion Etoposide plus thalidomide as maintenance therapy is associated with a significantly longer PFS with tolerable toxicity for elderly patients with advanced NSCLC.

Key words: etoposide; thalidomide; advanced non-small cell lung cancer (NSCLC); maintenance therapy

Received: 28 November 2016
Revised: 19 December 2016
Accepted: 23 March 2017

Lung cancer is one of the most common causes of cancer death worldwide. It leads to more than 1 million deaths of patients each year. Non-small cell lung cancer (NSCLC) accounts for up to 85% of lung cancer cases, and 65%–70% of advanced stage cases are unsuitable for radical surgery or radiotherapy [1]. The median survival after currently used platinum-based first-line treatments is approximately 8 to 10 months [2]. With increasing life expectancy, there has been a notably elevated incidence of lung cancer worldwide, owing to the increasing cancer risk associated with age. Recently, the use of maintenance chemotherapy for NSCLC has been extensively investigated [3–4]. After four to six cycles of platinum-based first-line therapy, maintenance therapy for patients with NSCLC without disease progression can prolong the time to disease progression, improve quality of life, and ultimately prolong overall survival [5–6]. Etoposide is an important chemotherapeutic agent that is used to treat a wide spectrum of human cancers. The primary cytotoxic target for etoposide is topoisomerase II, a ubiquitous enzyme that regulates DNA by under- and overwinding to remove knots and tangles from the genome by generating transient double-stranded breaks in the double helix [7]. It has been used clinically for more than two decades and remains one of the most highly prescribed anticancer drugs in the world. Etoposide as maintenance chemotherapy conferred a benefit for survival in patients with NSCLC [8]. In recent years, etoposide combined with anti-angiogenic agents showed very promising activity for advanced NSCLC [7, 9].

Angiogenesis, the proliferation of new blood vessels, is necessary for tumors to grow. Thalidomide is an oral anti-

✉ Corresponding to: Xiaodong Xie. Email: doctor_xxd@163.com.

angiogenic agent, inhibiting angiogenesis mediated by vascular endothelial growth factor (VEGF), the activity of basic fibroblast growth factors, and microvessel formation in experimental models [10]. This anti-angiogenic activity is considered a contributing factor for its antitumor effects in multiple myeloma, although the mechanism is not yet fully understood. It also has a synergistic activity when combined with cytotoxic agents and potentially has wider therapeutic activity to small cell lung cancer (SCLC) [11].

We compared the effect between maintenance etoposide plus thalidomide and best supportive care in patients without disease progression after four to six cycles of platinum-based first-line chemotherapy.

Patients and methods

Patient selection

We reviewed the characteristics of patients diagnosed with advanced NSCLC between 2014 and 2016 in the General Hospital of Shenyang Military Region, China. Patients who met all the following inclusion criteria were enrolled in this trial: (1) age > 60 years, (2) histologically or cytologically proven advanced NSCLC, (3) detection of the wild-type *EGFR* gene, *ALK* gene fusion, or unknown gene mutation status, (4) four to six cycles of platinum-based first-line chemotherapy, (5) Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2, (6) no disease progression after first-line chemotherapy, according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, (7) life expectancy of longer than 8 weeks, (8) no past tyrosine kinase inhibitors (TKI) treatment, and (9) adequate organ function [absolute neutrophil count (ANC) ≥ 1500 /mm^3, platelet count ≥ 100 000 /mm^3, hemoglobin ≥ 9.0 g/L, total bilirubin level ≤ 1.5 mg/dL, aminotransferase ≤ 2-fold upper limit of normal (ULN), creatinine ≤ 1.5 mg/dL or creatinine clearance ≥ 60 mL/min, and adequate renal function (calculated creatinine clearance > 50 mL/min)].

The exclusion criteria were as follows: (1) brain metastases, (2) diagnosis of synchronous malignant tumors, (3) unable to undergo follow-up, (4) severe bacterial infection, (5) intolerable adverse reaction to first-line chemotherapy, and (6) pregnancy or breastfeeding.

Treatments

Sixty-four elderly patients with advanced NSCLC according to the different maintenance treatment methods were divided as receiving etoposide plus thalidomide therapy (treatment group) and the best supportive care (control group). Etoposide was administered orally, 50 mg daily, from day 1 to 14, and the thalidomide starting dose was 100 mg/day during chemotherapy, and if tolerated, increased to 150 mg/day at the end of chemotherapy for three weeks. Dose modifications were made according to the drug instructions or the attending physician's judgment.

The recent curative effect objective response rate (ORR) [complete response (CR) + partial response (PR)] was defined as the proportion of patients whose tumors shrunk to a certain size and remained this way for a certain period (four weeks). Disease control rates (DCRs) included CR + PR + stable disease (SD). The long-term observation index, progression-free survival (PFS), was defined as the time from beginning maintenance treatment until disease progression. Tumor evaluation was performed using computed tomography one month after the start of medication and then every two months according to RECIST, version 1.1. Toxicity profiles were assessed and graded according to the Common Terminology Criteria for Adverse Events, version 3.0.

Statistical analysis

The primary endpoint was PFS. Secondary endpoints were tumor response (ORR and DCR) rates and toxicity. Clinicopathological characteristics were compared between the two groups by using the chi-square test. PFS was estimated using Kaplan-Meier curves and compared using the log-rank test. All statistical analyses were performed using SPSS 20.0 software (IBM SPSS, Armonk, NY, USA). *P* values less than 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 64 cases were enrolled in the trial. According to the different maintenance treatment methods, 32 patients received etoposide plus thalidomide therapy (treatment group) and 32 received best supportive care (control group). In this trial, the smoking history, clinical stage, pathological type (squamous- or adenocarcinoma), ECOG score, and first-line chemotherapy curative effect had no statistical significance. Thus, all the patients were eligible for further analysis. At the initiation of therapy, the median age of the 64 eligible patients was 55 years. The baseline characteristics of the patients were balanced between the two groups (Table 1).

Efficacy assessment of maintenance therapy

During a median 12-month follow-up, 9 of the 32 patients in the treatment group and 22 of the 32 patients in the control group experienced progressive disease. The responses (ORR + DCR) were observed in a higher proportion of patients in the treatment group compared with those in the control group (Table 2).

A total of 64 patients with NSCLC were observed in

Table 1 Baseline characteristics of the patients in the two groups

Characteristic	Treatment (n = 32)	Control (n = 32)	χ^2	P
Gender			0.567	0.451
Male	19 (59.4%)	16 (50.0%)		
Female	13 (40.6%)	16 (50.0%)		
Smoking			0.259	0.611
Smoker	18 (56.3%)	20 (65.6%)		
Non-smoker	14 (43.8%)	12 (34.4%)		
ECOG			0.132	0.715
0–1	26 (81.3%)	27 (84.4.%)		
2	6 (18.8%)	5 (15.6%)		
Histology			0.804	0.669
Squamous	17 (53.1%)	19 (59.4%)		
Adeno	14 (43.8%)	11 (34.4%)		
Others	1 (3.1%)	2 (6.2%)		
Stage			0.259	0.611
IIIB	18 (56.3%)	20 (62.5%)		
IV	14 (43.8%)	12 (37.5%)		
After first-line			0.784	0.376
CR	0 (0)	0 (0)		
PR	9 (28.1%)	6 (18.8%)		
SD	23 (71.9%)	26 (81.2%)		

Table 2 Treatment response of patients in the two groups (n)

Groups	n	CR	PR	SD	PD	ORR (%)	DCR (%)
Treatment	32	3	7	13	9	10 (31.3%)	23 (71.9%)
Control	32	0	1	9	22	1 (3.1%)	10 (31.3%)
χ^2						1.010	0.784
P						0.315	0.252

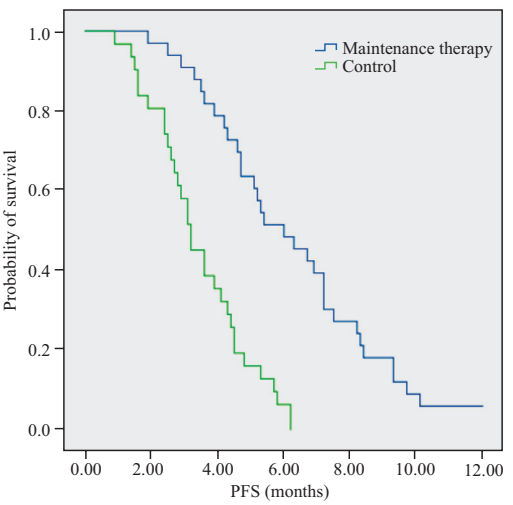


Fig. 1 PFS of the patients

our trial, and we compared the PFS between the two groups. We observed a trend of gained advantage in PFS in the treatment group compared with the control group. By the cutoff date, the median PFS in the treatment group was 6.0 months [95% confidence interval (CI) = 4.3–7.9 months], which was significantly longer than that in the control group (3.2 months, 95% CI = 2.6–3.8 months; χ^2=26.532, P = 0.001; Fig. 1).

Safety and tolerance

During our trial, we collected data regarding the severe adverse events. During maintenance therapy, the most frequently recorded grades 2–3 adverse events were hematologic abnormalities (20.3%), gastrointestinal toxicity (17.6%), and impaired liver function (9.7%). Etoposide was generally well tolerated. No grade 4 or higher adverse effects were recorded.

Discussion

Lung cancer is the principal cause of cancer-related death worldwide. The World Health Organization (WHO) classifies lung cancer into two subtypes: NSCLC and SCLC. NSCLC represents 85% of all lung cancer cases [1]. The first-line standard chemotherapy regimen for NSCLC is clear, but the effect has reached a plateau [12]. Maintenance therapy refers to the treatment period after completion of the initial standard chemotherapy for patients, and the purpose is to gain a maximum effect of tumor control after receiving continuous treatment. The treatment is not over until disease progression or intolerable toxicity occurs [5]. A non-randomized phase II study [9] have suggested that oral etoposide might be useful for treating extensive stage SCLC, especially in patients with advanced stage disease with poor prognosis. Patients aged > 65 years are likely to metabolize chemotherapeutic agents more slowly than younger patients, resulting in higher drug exposure levels and more serious adverse events [1]. Although pemetrexed is the recommended maintenance treatment for NSCLC, the need for intravenous therapy for long periods and the high cost are the reasons why most elderly patients with advanced lung cancer do not choose to receive pemetrexed. Etoposide has the advantages of being an orally administered agent with bioavailability. Etoposide involves topoisomerase II in the cell, inducing DNA double-strand breaks and early G2 phase blockage, but this effect disappears with reversal of etoposide [7]. The bioavailability of oral etoposide is 48%, and this may limit its clinical use as a palliative treatment, as some patients may be under-treated and others may have avoidable toxic effects. Single-agent oral etoposide as first-line chemotherapy and even maintenance therapy for SCLC prolongs the treatment time significantly [13]. A phase II trial found that the same dose of etoposide with sub-continuous administration had better effect than a single dose of therapy, and etoposide in the second-line

chemotherapy for refractory and relapsed patients could acquire 46% of the ORR [14]. As such, etoposide in our trial was administered in 50-mg doses, per os, on days 1–15 for three weeks. Cancers rely on angiogenesis for their growth and dissemination. Thalidomide has been shown to significantly inhibit tumor growth in mice injected with NSCLC cell lines, and early phase trial in humans has also indicated that this therapy was well tolerated and merited further investigation [15]. Although the precise antiangiogenic or resistance mechanisms of thalidomide are not yet fully understood, the possible reason is that tumor blood vasculature is already established in advanced NSCLC, VEGF may be superfluous, and angiogenesis is affected by other proangiogenic factors [16]. Other advantages of thalidomide are convenient oral administration and lower costs, and potentially beneficial anticachexia and immunomodulatory properties.

We believe that patients with a tumor response or stable disease might have more opportunity to benefit from agents such as etoposide and thalidomide. Morover, the new treatment is combined with standard chemotherapy in all patients at the start of chemotherapy, and then continues as maintenance. The PARAMOUNT study was the first proof of the effectiveness of maintenance therapy for advanced NSCLC [17]. The ASCO meeting in 2011 reported that the DCRs were 71.8% and 59.6%, respectively ($P = 0.009$) for the maintenance and placebo groups. PFS in the maintenance therapy group was 3.9 months, significantly longer than 2.6 months in the placebo group.

In our trial, the ORRs with etoposide plus thalidomide and best support were 31.3% and 3.1% ($\chi^2 = 0.010$, $P = 0.315$), and the DCRs were 71.9% and 31.3% ($\chi^2 = 0.784$, $P = 0.252$), respectively. The differences in ORRs and DCRs were not significant ($P > 0.05$) between the two groups. The median PFS with etoposide plus thalidomide and best support was 6.0 months (95% CI = 4.3–7.9 months) and 3.2 months (95% CI = 2.6–3.8 months), respectively. There was a significant difference in the PFS in the two groups ($P < 0.05$). Our trial indicated that etoposide plus thalidomide as maintenance therapy for advanced NSCLC after first-line chemotherapy significantly prolonged the PFS, with tolerable adverse reactions and convenient oral administration.

In conclusion, our data showed that etoposide plus thalidomide as maintenance therapy was associated with a PFS benefit in elderly patients with advanced NSCLC.

Acknowledgement

The authors would like to thank Liu Zhongzheng for his technical assistance.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Travis WD, Brambilla E, Burke AP, *et al*. WHO classification of tumours of the lung, pleura, thymus and heart. World Health Organization classification of tumours. 4th edition. France, Lyon: International Agency for Research on Cancer, 2015.
2. Masters GA, Temin S, Azzoli CG, *et al*. Systemic therapy for stage IV non-small-cell lung cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol, 2015, 33: 3488–3515.
3. Kolokotroni E, Dionysiou D, Veith C, *et al*. In silico oncology: quantification of the *in vivo* antitumor efficacy of cisplatin-based doublet therapy in non-small cell lung cancer (NSCLC) through a multiscale mechanistic model. PLoS Comput Biol, 2016, 12: e1005093.
4. Peters S, Adjei AA, Gridelli C, *et al*. Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol, 2012, 23 Suppl 7: vii56–64.
5. Rosell R, Karachaliou N. Lung cancer: Maintenance therapy and precision medicine in NSCLC. Nat Rev Clin Oncol, 2013, 10: 549–550.
6. Polo V, Besse B. Maintenance strategies in stage IV non-small-cell lung cancer (NSCLC): in which patients, with which drugs? Ann Oncol, 2014, 25: 1283–1293.
7. Zhu R, Wang Q, Zhu Y, *et al*. pH sensitive nano layered double hydroxides reduce the hematotoxicity and enhance the anticancer efficacy of etoposide on non-small cell lung cancer. Acta Biomater, 2016, 29: 320–332.
8. Petrioli R, Roviello G, Laera L, *et al*. Cisplatin, etoposide, and bevacizumab regimen followed by oral etoposide and bevacizumab maintenance treatment in patients with extensive-stage small cell lung cancer: A single-institution experience. Clin Lung Cancer, 2015, 16: e229–234.
9. Correale P, Botta C, Basile A, *et al*. Phase II trial of bevacizumab and dose/dense chemotherapy with cisplatin and metronomic daily oral etoposide in advanced non-small-cell-lung cancer patients. Cancer Biol Ther, 2011, 12: 112–118.
10. Kowalski TW, Sanseverino MT, Schuler-Faccini L, *et al.* Thalidomide embryopathy: Follow-up of cases born between 1959 and 2010. Birth Defects Res A Clin Mol Teratol, 2015, 103: 794–803.
11. Vasvari GP, Dyckhoff G, Kashfi F, *et al.* Combination of thalidomide and cisplatin in an head and neck squamous cell carcinomas model results in an enhanced antiangiogenic activity *in vitro* and *in vivo*. Int J Cancer, 2007, 121: 1697–1704.
12. Soda M, Choi YL, Enomoto M, *et al*. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature, 2007, 448: 561–566.
13. Young RJ, Tin AW, Brown NJ, *et al*. Analysis of circulating angiogenic biomarkers from patients in two phase III trials in lung cancer of chemotherapy alone or chemotherapy and thalidomide. Br J Cancer, 2012, 106: 1153–1159.
14. Johnson DH, Greco FA, Strupp J, *et al*. Prolonged administration of oral etoposide in patients with relapsed or refractory small-cell lung cancer: a phase II trial. J Clin Oncol, 1990, 8: 1613–1617.
15. Lee SM, Hackshaw A. A potential new enriching trial design for selecting non-small-cell lung cancer patients with no predictive biomarker for trials based on both histology and early tumor response: further analysis of a thalidomide trial. Cancer Med, 2013, 2: 360–366.

16. Devery AM, Wadekar R, Bokobza SM, *et al*. Vascular endothelial growth factor directly stimulates tumour cell proliferation in non-small cell lung cancer. Int J Oncol, 2015, 47: 849–856.
17. Paz-Ares LG, Altug S, Vaury AT, *et al*. Treatment rationale and study design for a phase III, double-blind, placebo-controlled study of maintenance pemetrexed plus best supportive care versus best supportive care immediately following induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small cell lung cancer. BMC Cancer, 2010, 10: 85.

DOI 10.1007/s10330-016-0207-7
Cite this article as: Ge YN, Zheng ZD, Liu ZZ, *et al*. Effect of etoposide plus thalidomide as maintenance therapy on progression-free survival of elderly patients with advanced non-small cell lung cancer. Oncol Transl Med, 2017, 3: 103–107.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0205-5

June 2017, Vol. 3, No. 3, P108–P110

ORIGINAL ARTICLE

The use of aprepitant and palonosetron in preventing chemotherapy-related nausea and vomiting inlung cancer patients

Shuxian Qu (✉), Zhendong Zheng (Co-first author), Zhaozhe Liu, Jianing Qiu, Xiaodong Xie (✉)

Department of Oncology, The General Hospital of Shenyang Military Region, Shenyang 110840, China

Abstract

Objective The aim of this study was to explore the clinical efficacy and toxicity of a combination aprepitant and palonosetron hydrochloride therapy in preventing chemotherapy-induced nausea and vomiting associated with a cisplatinum-based regimen in patients with lung cancer.

Methods Sixty-eight patients with lung cancer were randomly assigned to receive either aprepitant plus palonosetron hydrochloride (group A, n = 38) or tropisetron (group B, n = 30). Acute (0–24 h) and delayed (2–5 d) emetic episodes, nausea, vomiting, constipation, and dizziness were compared between the two groups in the five days following cisplatinum-based chemotherapy.

Results Group A had a higher complete control rate for both acute and delayed emetic episodes than Group B (36.8% vs. 13.3% and 31.6% vs. 13.3%, respectively; $P < 0.05$ for both). There was no significant difference in the constipation rate between the two groups.

Conclusion Aprepitant combined with palonosetron hydrochloride is active and well tolerated in both acute and delayed emetic episodes in patients with lung cancer treated by a cisplatinum-based regimen.

Received: 28 Novembver 2016
Revised: 10 December 2016
Accepted: 25 December 2016

Key words: aprepitant; palonosetron hydrochloride capsule; cisplatinum; tropisetron

Chemotherapy-induced nausea and vomiting (CINV) is a common and debilitating side effect of chemotherapy. Particularly high emetogenic chemotherapy drugs, such as cisplatin, can cause electrolyte imbalance, dehydration, and other complications [1]. CINV can also reduce the effects of chemotherapy, as well as patient compliance. The most common antiemetic drugs are the first generation 5-Hydroxytryptamine 3 (5-HT3) serotonin receptor antagonists [2–3]. However, these drugs have poor efficacy for the delayed nausea and vomiting frequently caused by cisplatin, because of their short half-lives. In this study, aprepitant and palonosetron capsules were administered to patients with lung cancer, receiving platinum-based chemotherapy from May 2015 to May 2016 to prevent chemotherapy-related nausea and vomiting, and the effects on CINV were examined.

Materials and methods

Patient information

The study included 68 patients, aged from 38 to 69 years, with a histopathological diagnosis of primary lung cancer. The patients were randomly divided into either an aprepitant and palonosetron group (Group A, n = 38) or a tropisetron group (Group B, n = 30), using prospective controlled study methods. All patients met the following inclusion criteria: Zubrod-ECOG-WHO score < 2, no brain metastases or gastrointestinal obstructions, no 5-HT3 receptor antagonist medication contraindications, no pregnant or breast-feeding women, and no indications preventing the administration of other antiemetic drugs. The two groups of patients had no statistically significant differences in baseline characteristics such as age, gender, chemotherapy, history of previous chemotherapy, or previous surgeries.

✉ Correspondence to: Shuxian Qu. Email: qushuxian2010@163.com; Xiaodong Xie. Email: doctor_xxd@163.com

Chemotherapy

Cisplatin (75 mg/m^2) was administered on the first day of a 21-day chemotherapy cycle. Other chemotherapeutic agents were provided according to the patient's normal regimen. Hydration was administered during chemotherapy to prevent renal toxicity.

Study drug

For Group A, patients received 125 mg aprepitant capsules (Novartis, USA) and intravenous injection of 0.25 mg palonosetron (Shandong Qilu Pharmaceutical Co., China) 30 min before chemotherapy. On days 2–4, patients took 80 mg aprepitant for oral.

For Group B, a 5 mg tropisetron sodium chloride injection (Shandong Qingfeng, China) was administered 30 min before chemotherapy, as well as on days 2 and 3. If vomiting occurred more than three times within a 24 h period, 5 mg dexamethasone was administered intravenously

Primary outcomes

Appetite, nausea, vomiting time, and severity of vomiting were recorded for five days following chemotherapy. Adverse reactions of the study drugs, such as headache, dry mouth, constipation, and facial flushing, were also recorded.

Evaluation criteria

All toxicities were graded using the Common Toxicity Criteria. Complete control (CR) rates (no emesis, no rescue) were analyzed for an acute (24 h after chemotherapy) and delayed (2–5 days after chemotherapy) period. Emetic episodes were scored on a scale of 0–III (0, CR); I, partial control (PR); II–III, invalid (SD)]. Remission was calculated as follows:

CR rate = complete response / total cases × 100%;

Effective control of acute vomiting rate = (number of cases + full control section controls the number of cases) / total cases × 100%.

Statistical analysis

SPSS v. 15.1 was used for statistical analysis. The Chi-square test was used for data comparison between groups. A P-value < 0.05 was considered to indicate statistical significance.

Results

Appetite

Appetite was similar between Group A and Group B (Table 1).

Table 1 The comparison of control of appetite

Time	Group (n)	Degree				CR (%)	χ^2	P
		0	I	II	III			
0–24 h	A (38)	5	20	8	5	13.2	0	1.000
	B (30)	4	16	6	4	13.3		
2–5 d	A (38)	10	13	13	2	26.3	4.454	0.035
	B (30)	2	14	12	2	6.7		

Table 2 The comparison of control of nausea

Time	Group (n)	Degree				CR (%)	χ^2	P
		0	I	II	III			
0–24 h	A (38)	13	17	5	3	34.2%	3.897	0.048
	B (30)	4	14	8	4	13.3%		
2–5 d	A (38)	12	14	11	1	31.6%	4.541	0.033
	B (30)	3	13	12	2	10.0%		

Table 3 The comparison of control of vomiting

Time	Group (n)	degree				CR (%)	χ^2	P
		0	I	II	III			
0–2 4h	A (38)	14	16	7	1	36.8	4.760	0.029
	B (30)	4	11	10	5	13.3		
2–5 d	A (38)	12	14	11	1	31.6	4.541	0.033
	B (30)	3	15	10	2	13.3		

Nausea

The control of acute and delayed nausea in Group A was better than in Group B (P < 0.05; Table 2).

Control of vomiting

The CR rate in Group A was 36.8% for the acute period and 31.6% for the delayed period. The CR rate in Group B was 13.3% for the acute period and 13.3% for the delayed period. Acute nausea and delayed vomiting were improved in Group A compared to Group B (P < 0.05; Table 3).

Adverse reactions

As shown in Table 4, the adverse reactions of the study drugs were similar between the two groups, namely, head heaviness, headache, fatigue, and dry mouth. Most patients had mild reactions. Some reactions may not have been specific to the study drugs, as it was difficult to distinguish from the effects of the chemotherapy. We observed no cases in Group A where chemotherapy was discontinued because of adverse reactions from the antiemetics.

Table 4 The comparison of adverse reaction

Adverse reaction	Group A (38)		Group B (30)	
	n	incidence (%)	*n*	incidence (%)
Dizzy	3	7.8	2	6.7
Thirst	5	13.2	4	13.3
Headache	4	10.5	3	10.0
Weak	3	7.8	2	6.7
Mild fever	1	2.6	0	0
Constipation	2	5.2	1	3.3
Anxiety	2	5.2	1	3.3
Diarrhea	1	2.6	1	3.3

Discussion

Nausea, vomiting, and other gastrointestinal symptoms are common adverse effects of chemotherapies used for lung cancer. Antagonists of 5-HT3 work by blocking the 5-HT receptors on the vagal afferent nerve endings of the gastrointestinal mucosa[2–4]. Palonosetron is a new 5-HT receptor antagonist, with a half-life of about 40 h and a strong affinity for the 5-HT3 receptor (more than 100 times greater than first-generation 5-HT receptor antagonists) [3–4]. There are a number of multi-center clinical studies demonstrating that palonosetron has a strong and long-lasting antiemetic effect [4–6]. In this study, palonosetron capsules in oral form had good absorption and bioavailability, with the maximum plasma levels being equal to intravenous administration.

Aprepitant is cited in the National Comprehensive Cancer Network guidelines as the first neurokinin-1 receptor antagonist to treat CINV [7]. Substance P is widely distributed in neuropeptide nerve fibers. When the nerve is stimulated, a large amount of substance P will be released and promote numerous biological processes by binding to NK-1. Aprepitant has a stronger affinity to NK-1 than to the 5-HT receptor [7–9]; thus, aprepitant combined with a 5-HT3 receptor antagonist should be able to prevent CINV better by acting on multiple targets.

Previous studies have shown that palonosetron can improve delayed nausea and vomiting control rate comparing to tropisetron, but not acute nausea or vomiting control rate [4]. In this study, the combination of palonosetron and aprepitant had an obvious advantage compared with the tropisetron group in efficiency and control rate in acute nausea, vomiting, and delayed emesis. This advantage resulted from the double antagonism of 5-HT receptors and substance P [9–10]. We observed a lower incidence of severe nausea and vomiting in the group that received the combination of aprepitant and palonosetron. No patients in the aprepitant and palonosetron group required a discontinuation of chemotherapy because of nausea, vomiting, and weight loss. However, the tropisetron group had three patients postpone chemotherapy for three days because of vomiting. We will need to increase the sample size in further studies to confirm this advantage of vomiting grade reduction.

There were no significant differences between the two groups in terms of adverse reactions to the study drugs. Group A had no severe adverse reactions resulting from the long half-life of palonosetron. This suggests a good security when aprepitant is combined with palonosetron. In short, the combination of aprepitant and palonosetron has a significant preventative effect against CINV after chemotherapy with cisplatin. This is a safe, economical, and effective treatment with good prospects for clinical application, particularly in controlling delayed emesis.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Maehara T, Matsumoto K, Horiguchi K,*et al*. Therapeutic action of 5-HT3 receptor antagonists targeting peritoneal macrophages in post-operative ileus. Br J Pharmacol, 2015, 172: 1136–1147.
2. Chen LL, Wang YQ, Ouyang DS. Advances in chemotherapy antic tactic drugs. Cancer Med (Chinese), 2014, 4: 107–109.
3. Mori-Vogt S, Blazer M. Palonosetron for the prevention of chemotherapy-induced nausea and vomiting. Expert Rev Anticancer Ther, 2013,13: 919–936.
4. Navari RM. Palonosetron for the treatment of chemotherapy-induced nausea and vomiting. Expert Opin Pharmacother, 2014,15: 2599–2608.
5. Morrow GR, Schwartzberg L, Barbour SY, *et al*. Palonosetron versus older 5-HT3 receptor antagonists for nausea prevention in patients receiving chemotherapy: a multistudy analysis. J Community Support Oncol, 2014,12: 250–258.
6. Mirabile A, Celio L,Magni M, *et al*. Evaluation of an every-other-day palonosetron schedule to control emesis in multiple-day high-dose chemotherapy. Future Oncol, 2014,10: 2569–2578.
7. Jordan K, Gralla R, Rizzi G, *et al*. Efficacy benefit of an NK1 receptor antagonist (NK1RA) in patients receiving carboplatin: supportive evidence with NEPA (a fixed combination of the NK1 RA, netupitant, and palonosetron) and aprepitant regimens. Support Care Cancer, 2016,24: 4617–4625.
8. Kashef K,Green S, Zwaan CM,Kang HJ. Aprepitant for paediatric chemotherapy-induced nausea and vomiting-Authors' reply. Lancet Oncol, 2015,16: 260–261.
9. Kusagaya H, Inui N, Karayama M, *et al*. Evaluation of palonosetron and dexamethasone with or without aprepitant to prevent carboplatin-induced nausea and vomiting in patients with advanced non-small-cell lung cancer. Lung Cancer, 2015, 90: 410–416.
10. Hanawa S, Mitsuhashi A, Matsuoka A, *et al*. Efficacy of palonosetron plus aprepitant in preventing chemoradiotherapy-induced nausea and emesis in patients receiving daily low-dose cisplatin-based concurrent chemoradiotherapy for uterine cervical cancer: a phase II study. Support Care Cancer, 2016, 24: 4633–4638.

DOI 10.1007/s10330-016-0205-5
Cite this article as: Qu SX, Zheng ZD, Liu ZZ, *et al*. The use of aprepitant and palonosetron in preventing chemotherapy-related nausea and vomiting in lung cancer patients. Oncol Transl Med, 2017, 3: 108–110.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0206-6

June 2017, Vol. 3, No. 3, P111–P115

REVIEW ARTICLE

Research progression of PD-1/PD-L1 in non-small cell lung cancer

Xing Liu[1], Xiaodong Xie[2] (✉)

[1] Liaoning University of Traditional Chinese Medicine, Shenyang 110847, China
[2] Department of Oncology, General Hospital of Shenyang Military Region, Shenyang, 110016, China

Abstract

Lung cancer is the leading cause of cancer-related mortality worldwide. Despite great progress in the development of target agents, most people who do not harbor a mutation do not benefit from these agents. Immunotherapy, which stimulates the body's immune system to improve the anti-tumor immunity effect, is a new therapeutic method for non-small cell lung cancer (NSCLC). Programmed cell death 1 (PD-1) and its ligand (PD-L1) belong to the CD28/B7 immunoglobulin super-family and are co-stimulatory molecules that show negative regulation effects. Combined with its ligand, PD-1 can modulate the tumor micro-environment, enabling tumor cells to escape host immune surveillance and elimination and play a key role in the clinical significance of NSCLC. An increasing number of clinical trials have suggested that immune checkpoint inhibitors, including anti-PD-1 and anti-PD-L1 monoclonal antibodies, are beneficial and safe for NSCLC. Here, we review the brief history of PD-L1 as a biomarker, mechanism of action, and critical role of PD-1/PD-L1 in the treatment of NSCLC as well as the current research status and future directions.

Received: 28 November 2016
Revised: 8 February 2017
Accepted: 12 April 2017

Key words: lung cancer; programmed cell death 1; programmed cell death-ligand 1; immunotherapy

With the deterioration of human living environment, the incidence of cancer worldwide is increasing each year, and lung cancer has become one of the most common malignant tumors; its incidence and mortality rate rank first among all malignant tumors [1–2]. This is also true in China, where the annual mortality of lung cancer is approximately 456 people per million people [3], replacing liver cancer as the leading cause of cancer death. In recent years, the morbidity and mortality of lung cancer have steadily increased, seriously threatening the life and health of humans [4]. For non-small-cell lung cancer (NSCLC), accounting for 80%–85% of primary lung cancers, approximately 75% of patients are diagnosed in the middle and late stages. The average survival periods for these stages are 12.9 months and 3 years, while the 5-year survival rates 19% and 11%, respectively [5]. Current treatment methods for NSCLC mainly include surgery, chemotherapy, radiotherapy, and molecular targeted therapy. Surgical treatment remains the only curative treatment, but many patients with NSCLC cannot undergo surgery. In recent years, studies of cancer immunotherapy have increased after molecular targeted therapy was developed. Programmed cell death-1 (PD-1) and its ligand programmed cell death-ligand 1 (PD-L1) have been examined as targets of immune therapeutic drugs in clinical trials and showed good efficacy and tolerance [6], showing potential for treating many patients with advanced lung cancer.

PD-1/PD-L1 expression as a bio-marker in early studies

In 1992, the Japanese scholar Ishida [7] discovered PD-1 in a T cell hybridoma 2B4.11 of mouse apoptosis. Because this molecule could inactivate T cells, Ishida named the protein "programmed death 1". PD-1 is an immunoglobulin B7-CD28 family member composed of an extracellular region, hydrophobic transmembrane region, and intracellular segment. The intracellular segment contains an immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif (ITSM). However, subsequent studies did not confirm the direct relationship between PD-1 and

✉ Correspondence to: Xiaodong Xie. Email: doctor_xxd@163.com

programmed cell death. Many years later, researchers deleted PD-1 to observe the effect on autoimmune disease, which began to clarify the function of PD-1 [8]. Freeman [9] confirmed the binding of a novel B7 molecule with PD-1, which inhibited the proliferation of T cells and production of cytokines. This molecule was named PD-L1, and the activation of ITSM is closely related to the response activity of effector T cells. PD-1 can be expressed on activated CD4+ T cells, CD8+ T cells, B cells, natural killer T cells, monocytes, and dendritic cells. Francisco [10] showed that PD-1 is also expressed in regulatory T cells (Treg) and can promote the proliferation of Treg cells to inhibit the immune response.

PD-1/PD-L1 expression in tumor cells

PD-L1 is highly expressed in many malignant tumors, including NSCLC [11–12], melanoma [13], renal cell carcinoma [14], prostate cancer [15], breast cancer [16], and glioma [17]. Studies [18–19] have shown that PD-L1 is also expressed in tumor-infiltrating dendritic cells, tumor-infiltrating lymphocytes, and tumor-infiltrating macrophages. The expression of PD-L1 may occur through two mechanisms [20]. Oncogene tumor control, also known as the innate resistance of tumor cells, occurs through PI3K-AKT, EGFR, ALK/STAT3, and other signaling pathways to induce tumor cell expression of PD-L1. The expression of PD-L1 is continuous and independent of the inflammation reaction in the tumor microenvironment [21–23]. Tumor immunity is driven by the microenvironment of T cells, also known as adaptive immune resistance, while PD-L1 expression is induced by inflammatory signals produced by the anti-tumor immune response, a non-persistent process that can prevent the body from infection-induced and immune-mediated tissue damage [24]. In addition, Spranger [25] showed that in melanoma models, the up-regulation of PD-L1 was closely related to CD8 T cells and did not depend on oncogene signaling. These studies indicate which patients may benefit from immune checkpoint blockade, the future studies are needed to clarify the role of these two mechanisms in different tumors. Notably, Butte [26] showed that PD-L1, in addition to binding PD-1, binds CD-80 (B7-1) to activate T cell surface binding, and then CD-80 transfers a negative regulation signal as a receptor rather than as a ligand.

PD-1/PD-L immune regulation

Mechanism of PD-1/PD-L on cellular immune regulation

PD-1 has two ligands, PD-L1 and PD-L2. PD-1 is located on the surface of activated T lymphocytes and can specifically recognize PD-L on antigen-presenting cells to activate the PD-1/PD-L1 pathway. This enables ITSM of the cytoplasmic region C of PD-1 to recruit and activate protein tyrosine phosphatase SHP-1 or SHP-2, thereby inhibiting the phosphorylation of ZAP-70 and preventing its binding to CD3ε, which can decrease the Ras-MAPK pathway, PCK pathway, and calcium-calmodulin pathway and result in decreased expression of activator protein-1, nuclear factor of activated T-cells, and nuclear factor-κB transcription factors. Thus, the proliferation, differentiation, and secretion of cytokines of T cells is inhibited. In addition, it can inhibit the proliferation of T cells by reducing the expression of the anti-apoptosis gene B-cell lymphoma-extra large and reduce T cell function by reducing the expression of GATA-3, Tbet, Eomes, and other transcription factors [27]. The PD-1/PD-L signaling pathway can also inhibit the phosphorylation of PI3K and serine/threonine kinases mediated by CD28, as well as weaken activation of the TCR/CD28 signal to immune cells [28]. Therefore, one of the main functions of the PD-1/PD-L signaling pathway is to inhibit the expression of transcription factors, thus inhibiting the activation of lymphocytes. These studies confirm that PD-1/PD-L signaling negatively regulates T cell immunity.

Mechanism of PD-1/PD-L on hum-oral immune regulation

Nishimura [29] found that the PD-1/PD-L signaling pathway not only participates in cellular immune regulation, but also regulates plasma cell production of antibodies. B cells can be activated by the direct identification of antigen peptides of antigen-presenting cells or by a combination of Tfh and co-stimulatory molecules. A previous study showed that high expression of PD-1 in Tfh cells and PD-L1 and PD-L2 in the germinal center of B cells resulted in upregulation [30] and that PD-1 had an inhibitory effect on cell immunity. These results indicate that the PD-1/PD-L signaling pathway affects the activation of B cells by inhibiting Tfh cells to reduce antibody generation. Additionally, this pathway can directly inhibit the phosphorylation of Igβ, Sy K, PLCγ2, and ERKI/2 by recruiting SHP-2 to inhibit the activation of B cells and secretion of cytokines [31]. Therefore, it is thought that PD-1 plays an important role not only in cellular immunity, but also in humoral immunity.

PD-1/PD-L1 closely related to immune evasion of lung cancer

Gene mutations that have been detected in NSCLC include those in EGFR, KRAS, and ALK, among others. Patients with KRAS mutations exhibit higher expression of PD-1 compared to those with wild-type KRAS. Additionally, the expression of PD-L1 protein is higher in patients with EGFR mutations and ALK gene

rearrangement [32]. Patients with expression of PD-1 and PD-L1 also have different clinical manifestations. Patients expressing PD-1 are typically smokers, male, and have adenocarcinoma, which is consistent with the clinical characteristics of patients with KRAS gene mutations. Those expressing PD-L1 are typically non-smoking, female, and have adenocarcinoma, which agrees with the clinical features of patients with EGFR mutations [33]. The EGFR signaling pathway, by inducing PD-L1 expression, can help tumors escape the body's anti-tumor immune response, and EGFR-TKIs can block the PD-1 signaling pathway, reduce the expression of PD-L1, and improve the overall survival rate of patients [34]. In patients with EGFR mutations and high expression of PD-L1, the sensitivity of gefitinib and erlotinib are increased, which may be because EGFR inhibitors down-regulate the expression of PD-L1 [33]. Lin [35] found that in lung adenocarcinoma patients with EGFR mutation, more than 50% showed abnormal expression of PD-L1. Among 56 patients with EGFR mutations who underwent EGFR-TKI treatment (positive rates of PD-L1 and PD-1 were 53.6% and 32.1%), PD-L1-positive patients showed a better disease control rate, longer progression-free survival, and better overall survival, suggesting that PD-L1 is a useful biomarker for EGFR-TKI.

Over-expression of the PD-L1 gene can lead to NSCLC immune escape, and recently numerous clinical studies have demonstrated that inhibiting the PD-L1 or PD-1 gene can be used in the treatment of NSCLC [36]. Blocking both PD-1 and PD-L1 can result in a good response rate for NSCLC [37]. Therefore, in the future, immune monitoring inhibitors are likely to become an important aspect of conventional treatment of NSCLC.

Potential of PD-1/PD-L1 as a target molecule in clinical therapy

Targeted immunotherapy for blocking PD-1/PD-L1 was widely evaluated in the oncology field in 2013. In NSCLC, most studies of immunotherapy involved allogeneic tumor vaccine, autologous cell therapy, and T-cell modulators [38]; multiple II or III phase clinical trials have also been conducted [39]. In 2006, the US FDA approved a humanized anti-human PD-1 monoclonal antibody for clinical treatment studies of cancer and infectious diseases. Following the listing of anti-CTLA4 antibody, anti-PD-1 antibody entered clinical trials in October 2011 to evaluate drug safety, adverse drug reactions, and survival benefit, among other factors.

Brahmer [40] reported a dose escalation phase I clinical trial (NCT00730639) of 127 patients undergoing re-treatment for NSCLC. Twelve cycles of anti-PD-1 antibody (Nivolumab) (1–10 mg/kg, 1–2 weeks) were administered to explore the safety and efficacy of anti-Nivolumab treatment of lung squamous cell carcinoma and NSCLC. The study showed that anti-PD-1 antibody had good safety and that the overall survival of re-treated patients with advanced NSCLC was significantly improved. Gettinger [41] reported another randomized phase III clinical trial (NCT01673867) of 574 patients with first-line treatment failure NSCLC. Following 1:1 random grouping, one group was treated with anti-PD-1 antibody (3 mg/kg, 1–12 weeks), while the other group received docetaxel until disease progression or the patient could no longer tolerate the toxic side effects to compare the overall survival of these two second-line treatment options. The study showed that overall survival was longer with nivolumab than with docetaxel. The median overall survival was 12.2 months (95% confidence interval [CI], 9.7–15.0) among 292 patients in the nivolumab group and 9.4 months (95% CI, 8.1–10.7) among 290 patients in the docetaxel group (hazard ratio for death, 0.73; 96% CI, 0.59–0.89; $P = 0.002$). At 1 year, the overall survival rate was 51% (95% CI, 45–56) with nivolumab versus 39% (95% CI, 33–45) with docetaxel. With additional follow-up, the overall survival rate at 18 months was 39% (95% CI, 34–45) with nivolumab versus 23% (95% CI, 19–28) with docetaxel. These results indicate that among patients with advanced NSCLC that had progressed during or after platinum-based chemotherapy, overall survival was longer with nivolumab than with docetaxel. Clinical trials of anti-PD-L1 antibodies for treating NSCLC are mostly in the phase I/II/III to evaluate the best dose and adverse reactions [42]. Spigel [43] reported a multicenter, dose escalation phase I clinical trial (NCT01375842) of 53 patients with locally advanced/metastatic NSCLC (who had undergone surgery or radiotherapy) given different doses of anti-PD-1 drugs (1, 3, 10, or 20 mg/kg, 1–3 weeks) for 1 year to study the clinical activity, safety, and bio-markers of anti-PD-L1 antibody (MPDL3280A) for NSCLC. The study showed that the median duration of treatment was 106 days (range 1–324 days), objective response rate was 24%, and progression-free survival at 24 weeks was 48%. The curative effect of MPDL3280A was correlated with PD-L1 expression. The adverse reaction rate of 3/4 was 34% (including pericardial transfer, dehydration, breathing the difficulty, fatigue, diarrhea, and pneumonia). In addition, MPDL3280A treatment of partial NSCLC patients showed delayed efficacy [44].

As additional studies are conducted, the role of PD-1/PD-L in immune regulation and the study, prevention, diagnosis, and treatment of various types of clinical diseases will be clarified [45]. Anti-PD-L1 antibody testing will reveal which population will benefit from treatment, biomarkers, and the presence of a synergistic anti-tumor effect between immune drugs [46]. As a negative synergistic stimulation signal, PD-1/PD-L plays an important role in the immune response regardless of breadth or depth,

as well as plays a critical role in immune tolerance and immune injury. PD-1/PD-L is a promising target molecule for tumor immunotherapy.

Summary and outlook

Immune drug therapy as a new treatment regimen for NSCLC (in addition to chemotherapy, radiotherapy, and molecular targeted therapy, etc.) can improve the response rate, reduce side effects, has economic advantages, and be used as an outpatient drug. Combination chemotherapy based on such drugs or single drug treatment is not only suitable for elderly patients with poor organ function and poor physical fitness who refuse chemotherapy, but also can be used as a palliative treatment [47]. Additional studies are needed to verify the feasibility of PD-1/PD-L1 as biomarkers and validate the clinical value of combination PD-1/PD-L1 inhibitors and other therapies, as well as to develop "PD-1/PD-L1 testing guidelines" to determine which population is suitable for tumor immunotherapy, optimize the immune therapy, and avoid the damage caused by immune toxicity to patients. When the immune drug is used, the length of treatment, use alone or in combination, groups that will show the greatest benefit, drug resistance mechanisms, and how to overcome drug resistance require further evaluation.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. He J, Chen W. 2012 Chinese cancer registry annual report. Beijing: Military Medical Science Press. 2012.
2. Agulló-Ortuño MT, López-Ríos F, Paz-Ares L. Lung cancer gernomic signatures. J Thorac Oncol, 2010, 5: 1673–1691.
3. Zhao L, Wang L. Advancements in radiotherapy for lung cancer in China. Oncol Transl Med, 2015,1: 5–14.
4. Chen WQ, Zhang SW, Zou XN. The estimates and popular trend research on lung cancer death in China. Chin J Lung Cancer (Chinese), 2010, 13: 5.
5. Sher DJ, Koshy M, Liptay MJ, *et al*. Influence of conformal radiotherapy technique on survival and chemoradiotherapy for patients with stage III non-small cell lung cancer in the National Cancer Data Base. Cancer, 2014, 120: 2060–2068.
6. Shien K, Papadimitrakopoulou VA, Wistuba II. Predictive biomarkers of response to PD-1/PD-L1 immune checkpoint inhibitors in non-small cell lung cancer. Lung Cancer, 2016, 99: 79–87.
7. Ishida Y, Agata Y, Shibahara K, *et al*. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J, 1992, 11: 3887–3895.
8. Dong H, Zhu G, Tamada K, *et al*. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med, 1999, 5: 1365–1369.
9. Keir ME, Butte MJ, Freeman GJ, *et al*. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol, 2008, 26: 677–704.
10. Francisco LM, Salinas VH, Brown KE, *et al*. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med, 2009, 206: 3015–3029.
11. Velcheti V, Schalper KA, Carvajal DE, *et al*. Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest, 2014, 94: 107–116.
12. Boland JM, Kwon ED, Harrington SM, *et al*. Tumor B7-H1 and B7-H3 expression in squamous cell carcinoma of the lung. Clin Lung Cancer, 2013, 14: 157–163.
13. Algazi AP, Tsai KK, Shoushtari AN, *et al*. Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-L1 antibodies. Cancer, 2016, 122: 3344–3353.
14. Kammerer-Jacquet SF, Crouzet L, Brunot A, *et al*. Independent association of PD-L1 expression with non-inactivated VHL clear cell renal cell carcinoma–A finding with therapeutic potential. Int J Cancer, 2017, 140: 142–148.
15. Goltz D, Holmes EE, Gevensleben H, *et al*. CXCL12 promoter methylation and PD-L1 expression as prognostic biomarkers in prostate cancer patients. Oncotarget, 2016, 7: 53309–53320.
16. Soliman H, Khalil F, Antonia S. PD-L1 expression is increased in a subset of basal type breast cancer cells. PLoS One, 2014, 9: e88557.
17. Liu Y, Carlsson R, Ambjorn M, *et al*. PD-L1 expression by neurons nearby tumors indicates better prognosis in glioblastoma patients. J Neurosci, 2013, 33: 14231–14245.
18. Mu CY, Huang JA, Chen Y, *et al*. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol, 2011, 28: 682–688.
19. Taube JM, Klein A, Brahmer JR,*et al*. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res, 2014, 20: 5064–5074.
20. Pardoll DM.The blockade of immune check points in cancer immunotherapy. Nat Rev Cancer.2012, 12(4): 252-264.
21. Parsa AT, Waldron JS, Panner A, *et al*. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med, 2007, 13: 84–88.
22. Akbay EA, Koyama S, Carretero J, *et al*. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov, 2013, 3: 1355–1363.
23. Marzec M, Zhang Q, Goradia A, *et al*. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proc Natl Acad Sci U S A, 2008, 105: 20852–20857.
24. Taube JM, Anders RA, Young GD, *et al*. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med, 2012, 4: 127ra37.
25. Spranger S, Spaapen RM, Zha Y, *et al*. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med, 2013, 5: 200ra116.
26. Butte MJ, Keir ME, Phamduy TB, *et al*. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity, 2007, 27: 111–122.
27. Nurieva R, Thomas S, Nguyen T, *et al*. T cell tolerance or function is determined by combinatorial costimulatory signals. EMBO J, 2006, 25: 2623–2633.
28. Parry RV, Chemnitz JM, Frauwirth KA, *et al*. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell

Biol, 2005, 25: 9543–9553.
29. Nishimura H, Okazaki T, Tanaka Y, *et al*. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science, 2001, 291: 319–322.
30. Lee SK, Rrgby RJ, Zotos D, *et al*. B cell priming for ex-trafollicular antibody responses requires Bcl-6 expression by T cells. J Exp Med, 2011, 208: 1377–1388.
31. Keir ME, Butte MJ, Freeman GJ, *et al*. PD-1 and its li-gands in tolerance and immunity. Annu Rev Immunol, 2008, 26: 677–704.
32. Gao BB, Li DQ. The research progress and prospects of PD-1/PD-L1 in non-small cell lung cancer. J Clin Pathol, 2015, 35: 1189–1194.
33. D'Incecco A, Andreozzi M, Ludovini V, *et al*. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer, 2015, 112: 95–102.
34. Akbay EA, Koyama S, Carretero J, *et al*. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov, 2013, 3: 1355–1363.
35. Lin C, Chen X, Li M, *et al*. Programmed death-ligand 1 expression predicts tyrosine kinase inhibitor response and better prognosis in a cohort of patients with epidermal growth factor receptor mutation-positive lung adenocarcinoma. Clin Lung Cancer, 2015, 16: e25–35.
36. Tang L BJ, Chung CS, Lomas-Neira J, *et al*. Active players in resolution of shock/sepsis induced indirect lung injury: immunomodulatory effects of Tregs and PD-1. J Leukoc Biol, 2014, 4MA1213–1647RR.
37. Spigel DR SM. Rationale for chemotherapy, immunotherapy, and checkpoint blockade in SCLC: beyond traditional treatment approaches. J Thorac Oncol, 2013, 8: 587–598.
38. Shah S, Ward JE, Bao R, *et al*. Clinical response of a patient to anti-PD-1 immunotherapy and the immune landscape of testicular germ cell tumors. Cancer Immunol Res, 2016, 4: 903–909.
39. Sorensen SF, Demuth C, Weber B, *et al*. Increase in soluble PD-1 is associated with prolonged survival in patients with advanced EGFR-mutated non-small cell lung cancer treated with erlotinib. Lung Cancer, 2016, 100: 77–84.
40. Gettinger SN, Horn L, Gandhi L, *et al*. Overall survival and long-term safety of nivolumab (anti-programmed death 1 antibody, BMS-936558, ONO-4538) in patients with previously treated advanced non-small-cell lung cancer. J Clin Oncol, 2015, 33: 2004–2012.
41. Borghaei H, Paz-Ares L, Horn L, *et al*. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med, 2015, 373: 1627–1639.
42. Robert L, Harview C, Emerson R, *et al*. Distinct immunological mechanisms of CTLA-4 and PD-1 blockade revealed by analyzing TCR usage in blood lymphocytes. Oncoimmunology, 2014, 3: e29244.
43. Spigel DR, Chaft JE, Gettinger SN, *et al*. Clinical activity and safety from a phase II study (FIR) of MPDL3280A (anti-PDL1) in PD-L1–selected patients with non-small cell lung cancer (NSCLC). J Clin Oncol, 2015, 33(suppl): abstr 8028.
44. Sunshine J, Taube JM. PD-1/PD-L1 inhibitors. Curr Opin Pharmacol, 2015, 23: 32–38.
45. Zitvogel L,Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology, 2012, 1: 1223–1225.
46. Kong YC, Flynn JC. Opportunistic autoimmune disorders potentiated by immune-checkpoint inhibitors anti-CTLA-4 and anti-PD-1. Front Immunol, 2014, 5: 206.
47. Takada K, Toyokawa G, Okamoto T, *et al*. An immunohistochemical analysis of PD-L1 protein expression in surgically resected small cell lung cancer using different antibodies and criteria. Anticancer Res, 2016, 36: 3409–3412.

DOI 10.1007/s10330-016-0206-6
Cite this article as: Liu X, Xie XD. Research progression of PD-1/PD-L1 in non-small cell lung cancer. Oncol Transl Med, 2017, 3: 111–115.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0210-0

June 2017, Vol. 3, No. 3, P116–P126

ORIGINAL ARTICLE

The role of the HGF/c-Met signaling pathway in crizotinib-induced apoptosis in lung cancer with c-Met amplification*

Shaozhang Zhou[1], Zhixin Dong (Co-first author)[2], Jinyi Lv[2], Aiping Zeng[1], Huilin Wang[1], Ruiling Ning[1], Xiangqun Song[1] (✉)

[1] The Second Department of Chemotherapy, The Affiliated Tumor Hospital, Guangxi Medical University, Guangxi 530021, China
[2] Guangxi Medical University Graduate School, Guangxi 530021, China

Abstract

Objective This study aimed to study the role of the HGF/c-Met signaling pathway in crizotinib-induced apoptosis of various lung adenocarcinoma cell lines and xenograft tumor models.

Methods *In vitro*, H2228, H1993, and A549 cells were treated with crizotinib. The inhibition of proliferation was quantitated by a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Apoptosis was quantified by flow cytometry. Expression of key proteins of the HGF/c-Met signaling pathway was examined by western blotting. *In vivo*, H1993 and A549 tumor cell xenograft models were established. Immunohistochemical analysis was used to determine protein expression of HGF and c-MET and the amount of phospho-c-MET (p-c-Met). Real-time quantitative polymerase chain reaction (PCR) was applied to examine the messenger RNA (mRNA) expression of c-MET and serine/ threonine protein kinase (AKT). The expression and activation of the key proteins were evaluated by western blotting.

Results *In vitro*, the growth of H1993, H2228, and A549 cells was inhibited after crizotinib treatment for 72 h. Apoptotic rates of H1993 and H2228 cells increased with the crizotinib concentration and exposure time. *In vivo*, the growth-inhibitory rate of crizotinib for H1993 xenografts was 72.3%. Positive expression rates of HGF and c-MET in H1993 xenografts were higher than those in A549 xenografts; the p-c-MET amount was the largest in H1993 xenograft control but the lowest in the H1993 xenograft with crizotinib treatment. The mRNA expression levels of c-MET and AKT in H1993 xenografts were higher than those of A549 xenografts. The protein levels of c-MET, AKT, and extracellular regulated protein kinases (ERK) in H1993 xenografts were higher than those in A549 xenografts; the p-AKT amount was higher in H1993 xenograft control than in A549 xenografts; the largest amount of p-c-MET was detected in H1993 xenograft control; the amount of p-ERK was the lowest in the H1993 xenograft with crizotinib treatment.

Conclusion The HGF/c-Met signaling pathway may mediate crizotinib-induced apoptosis and inhibition of proliferation of lung adenocarcinoma cells.

Received: 4 December 2016
Revised: 25 December 2016
Accepted: 9 February 2017

Key words: HGF/c-MET signaling pathway; H1993 cells; H2228 cells; crizotinib; apoptosis

Lung cancer is the leading cause of cancer-related deaths worldwide, and nearly 80% of patients with non-small cell lung cancer (NSCLC) receive the diagnosis at an advanced stage. The efficacy of platinum-based doubled regimens is limited, with progression-free survival and median survival of only 4–5 months and 8–9 months, respectively. In recent years, precision medicine, which involves diagnosis and treatment based on a patient's biogenetic information, has been increasingly applied. In patients with NSCLC containing *EGFR* mutations or *ALK* rearrangements, targeted therapies represent the standard of care, with superior efficacy and improved

✉ Correspondence to: Xiangqun Song. E-mail: xiangquns@163.com
* Supported by grants from the National Natural Sciences Foundation of China (No. 81060188 and No. 81260351) and Guangxi Sciense & Technology Development Funds (No. 2015139 and No. 201017).

tolerability, compared with chemotherapy. These two genetic alterations are relatively common and are found in tumors of approximately 15% and 5% of patients with NSCLC, respectively. Extensive profiling efforts have identified molecular drivers in subsets of NSCLC.

The proto-oncogene *c-Met* encodes receptor tyrosine kinase *c-Met*, which belongs to the class of transmembrane receptors with autophosphorylation activity [1]. After a specific natural ligand called hepatocyte growth factor (HGF) binds to c-Met's extracellular domain, *c-Met* can undergo dimerization and autophosphorylation and subsequently activate downstream signaling pathways such as PI3K/AKT, Ras-Rac/Rho, MAPK/MAP, and STAT3/survivin signaling cascades [2]. In some cases, abnormal activation of HGF/c-Met signaling contributes to oncogenesis and tumor progression in a variety of cancers and promotes invasiveness that is strongly linked to tumor metastasis [3]. The most common aberrant types of c-Met genes include *c-Met* point mutations and amplification. *c-Met* amplification is present in tumors of approximately 2.2% and 5% of NSCLC patients untreated or with failed treatment with EGFR tyrosine kinase inhibitors (EGFR-TKIs), respectively [4]. Preclinical analysis has shown that growth and survival of lung cancer cell lines with *c-Met* primary amplification depend on activation of the HGF/c-Met signaling pathway [5]. Targeting *c-Met* may be a promising strategy for lung cancer treatment, but there are few relevant studies. Crizotinib (PF-02341066), an inhibitor of tyrosine kinase activity of *c-Met*, ALK, and ROS-1, has been shown to be effective in the treatment of patients with EML4-ALK- or ROS-1-positive NSCLC and was approved by the Food and Drug Administration in August 2011 and March 2016, respectively. NSCLC harboring strongly amplified *c-Met* or *c-Met* exon 14 alterations may represent independent c-Met phenotypes and responses to crizotinib [6–7]. National Comprehensive Cancer Network (NCCN) Guideline Version 2.2016 NSCLC expanded the indications for the strong *c-Met* amplification or *c-Met* exon 14 skipping mutation and ROS-1 rearrangements. Nonetheless, the role of the HGF/c-Met signaling pathway in crizotinib-induced apoptosis in NSCLC with *c-Met* amplification has yet to be determined.

In this study, we evaluated the role of the HGF/c-Met signaling pathway in crizotinib-induced apoptosis of various lung adenocarcinoma cell lines and xenograft tumor models and analyzed the possible regulatory mechanisms with the goal of providing a scientific basis for crizotinib use in the treatment of primary or secondary *c-Met* amplification-positive NSCLC as well as experimental data on its efficacy and safety.

Materials and methods

Materials

Cell lines

c-Met amplification-positive human lung adenocarcinoma cell line H1999 and E*ML4-ALK*-positive human lung adenocarcinoma cell line H2228 (*ALK* rearrangement variant type V3) were acquired from the American Type Culture Collection. The A549 cell line was provided by the Experimental Center of The Affiliated Tumor Hospital of Guangxi Medical University.

Mice

Male specific-pathogen-free (SPF), 3- to 5-week-old, BALB/C nude mice (18–22 g, n = 40) were purchased from the Animal Experimental Center of Guangxi Medical University (Permission No. for Laboratory Animals: SYXK GUI 2014-0002). The animals were housed in autoclaved Micro-Isolater clear plastic cages, with a ventilation rate of 10 to 15 air changes per hour, at 20°C, 50% relative humidity, and in a light/dark cycle of 12/12 h, with free access to standard rodent food pellets and water. All animal experimentation was performed according to institutional guidelines and with approval of a local institutional review board.

Reagents and instruments

Crizotinib was acquired from Cell Signaling Technology (USA). The RPMI-1640 medium, fetal bovine serum (FBS), and trypsin were purchased from Gibco (USA). A 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution and dimethyl sulfoxide (DMSO) were purchased from Amresco (USA). The Annexin-V-PE/7AAD apoptosis detection kit and the Bicinchoninic acid (BCA) kit were purchased from BD (USA) and Merck (China), respectively. The SP Immunohistochemical (IHC) Kit was acquired from Boosen Biological Technology (China). Diaminobenzidine (DAB) was purchased from Hill-bridge Company (China). The Total RNA Small Amount Preparation Kit was purchased from Axygen Corelle Life Science Company (China). The cDNA Synthesis Kit was acquired from Bao Biological Engineering Company (China), and the Quantitative PCR Kit and Phosphatase Inhibitors Cocktail Tablets were purchased from Roche (Switzerland). The BCA Protein Assay Kit was acquired from Beyotime Biotech Company (China). HGF, c-MET, phosphorylated c-MET (p-c-MET; Tyr1003), AKT, p-AKT (Ser473), ERK, p-ERK (Thr202/Thr204), and β-actin antigen reagents and antibodies were purchased from Cell Signaling Technology (USA). Fluorescence type II antigen and antibody were purchased from Poly Biological Technology (China). Supersensitive ECL Chemical Luminescence Substrate was purchased from Boster Biological Engineering Company (China). The quantitative PCR instrument was acquired from Eppendorf (Germany). The JY-ECPT3000 electrophoresis

device was purchased from Liuyi instrument factory (China). Western blot equipment and a type 170-8170 protein imaging instrument were acquired from Bio-Rad Company (USA).

Methods

Cell culture

Cells were cultured in the RPMI 1640 medium supplemented with 10% of FBS at 37°C in a humidified atmosphere containing 5% of CO_2. The culture medium was removed every 2–3 days, and 1–2 mL of a trypsin-ethylene diamine tetraacetic acid (EDTA) solution was added. The flask was allowed to incubate at room temperature until the cells got detached. A fresh culture medium was added, and the solution was aspirated and dispensed into new culture flasks.

The MTT assay

Attached H1993, H2228, and A549 cells in culture were digested with 0.25% trypsin until they got detached. The cells were centrifuged, the supernatant was discarded, and the cells were resuspended in the complete growth medium. After the cell density was adjusted to 800 per mL, the cells were seeded in 96-well plates at 100 μL/well, and incubated overnight at 37°C and 5% CO_2. The cells were then exposed to 10, 30, 90, 270, or 810 nM crizotinib for 72 h. Absorbance at 490 nm (A490) was measured on a Multiskan Spectrum spectrophotometer. Each assay consisted of six replicate wells and was repeated at least three times independently. Inhibition of cell proliferation was calculated as follows: [1 - (A_{490} of experimental group -A_{490} of blank group) (A_{490} of control group - optical density of blank group)] × 100%.

Flow cytometric analysis of the effect of crizotinib on apoptosis

Attached H1993, H2228, and A549 cells in culture were digested with 0.25% trypsin and centrifuged. The supernatant was discarded, and the cells were resuspended in the complete growth medium. The cell densities were adjusted to 3×10^5/mL, 6×10^5/mL, and 10^6/mL, respectively, and the cells were seeded in 6-well plates at 2 mL/well. Crizotinib at 200, 300, and 300 nM was added to the medium of H1993, H2228, and A549 cells, respectively. The cells were cultured for 24, 48, or 72 h, and were harvested by incubation with 0.25% trypsin without EDTA, washed with phosphate-buffered saline (PBS), and centrifuged at 300 × *g* for 5 min. The cell pellets were resuspended in 100 μL of the Annexin-V-PE/7AAD labeling solution, and incubated for 15 min at room temperature in the dark. After addition of propidium iodide (PI) and fluorescein from the isothiocyanate (V-PE/7AAD) staining kit, the cells were resuspended in 400 μl of the Annexin-V-PE/7AAD labeling solution and then analyzed for fluorescence by flow cytometry.

Western blot analysis

Cells were washed twice with ice-cold PBS and then lysed in a solution containing phosphatase inhibitors cocktail tablets, phenylmethanesulfonyl fluoride (PMSF), and RIPA Lysis Buffer (100 mM), according to the manufacturer's protocol. The protein concentration in the cell lysates was measured using the BCA kit, and equal amounts of protein were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in an 8% or 10% gel. β-Actin served as an internal reference. The separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were then exposed to 5% bovine serum albumin (BSA) in PBS for 2 h at room temperature before overnight incubation at 4°C with primary antibodies. The membranes were incubated for 1 hour at room temperature with horseradish peroxidase-conjugated goat antibodies to rabbit, horse, or mouse IgG. According to the manufacturer's protocols, the c-MET antibody, p-c-MET (Tyr1003) antibody, AKT antibody, p-AKT (Ser473) antibody, ERK antibody, p-ERK (Thr202/Thr204) antibody, and the β-actin antibody were used at 1:1000, 1:1000, 1:2000, 1:2000, 1:1000, 1:2000, and 1:1000 dilutions, respectively. The membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat antibodies to rabbit or horse antibodies to mouse IgGThe membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat antibodies to rabbit or horse antibodies to mouse IgG, which were used at a 1:2000 dilution.

Animal grouping and treatment

Forty SPF BALB/C nude mice were randomly allocated to 5 groups on average: H1993 xenograft control (Group A), H1993 xenograft with crizotinib (Group B), A549 xenograft control (Group C), A549 xenograft with crizotinib (Group D), and healthy control (Group E). For the tumor inoculation model, subcutaneous tissue of the left axillary region from BALB/C mice was injected with the H1993 or A549 cell line at a concentration of 5×10^7 cells per 100–150 μL. The animal models were regarded as successful when the tumor size reached 200–600 mm^3 (day 0) in each mouse. Treatment groups consisted of control and crizotinib-administered (50 mg/kg of body weight, which was dissolved in 0.2 mL of distilled water) mice. Crizotinib was administered by oral gavage daily for 14 days; control animals received 0.2 mL of distilled water as vehicle. Weight, food intake, and tumor size of the mice were measured every 3 days from the day of crizotinib administration. Tumor volume was determined by caliper measurements of tumor length (L) and width (W) according to the formula $L \times W^2/2$. Tumor growth curves and inhibition rates were calculated. Euthanasia by cervical dislocation was performed 24 h after the last drug administration, and tumors were completely excised

for further analysis. Percentages of inhibition of tumor volume were measured on the final day of the experiment for drug-treated versus vehicle-treated mice and were calculated as follows: [($Control_{final\ day}$ - $Treated_{final\ day}$) / $Control_{final\ day}$].

IHC analysis

Sections of 4-μm thickness cut from formalin-fixed paraffin-embedded tissue blocks were deparaffinized in xylene and rehydrated in a graded series of aqueous ethanol solutions. Levels of HGF, c-MET, and p-c-MET in tumor tissues were measured by means of the SP kit. IHC staining results were assessed using the criteria of Fromowitz semiquantitative classification, where the total score = staining intensity score + proportion of positive cells score. Staining intensity was scored based on membrane staining intensity: 0 = no staining, 1 = faint yellow, 2 = pale brown, and 3 = dark brown. The proportions of positive cells were scored as follows: < 5% for score = 0, 5%–25% for score = 1, 26%–50% for score = 2, 51%–75% for score = 3, and > 75% for score = 4. The total score < 2 was defined as negative (-), 2–3 as weakly positive (+), 4–5 as moderately positive (++), and 6–7 as strongly positive (+++). Low and high expression were designated as "-" to + and ++ to +++, respectively.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from snap-frozen samples by means of the TRIzol Reagent (Invitrogen, USA). Complementary DNA was synthesized from 500 ng of total RNA using the cDNA Synthesis Kit (Bao Biological Engineering Company, China). The gene-specific primers for c-MET and AKT are listed in Table 1. The primers were synthesized by the Hua Da gene Polytron Technology Company (China). For normalization of the RNA quality and quantity, expression of β-actin in each sample was quantified. Messenger RNA (mRNA) expression of c-MET and AKT was determined using the SYBR Green-Based RT-PCR Kit and a quantitative RT-PCR system (Germany) under the following conditions: an initial denatur¬ation step of 95°C for 10 min, followed by 40 amplifica¬tion cycles involving denaturation at 95°C for 15 sec and annealing with elongation at 60°C for 1 min. Signals with a (maximal curvature) threshold cycle (Cq) value of > 35 were assumed to indicate no transcription of the target gene. A melting-curve analysis was conducted to monitor PCR product purity, and relative gene expression data were analyzed by the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta Cq = (Cq_{target\ gene} - Cq_{internal\ control})_{testing\ sample} - (Cq_{target\ gene} - Cq_{internal\ control})_{standard\ sample}$. One randomly selected sample served as a reference sample to calculate the relative quantity value.

Table 1 Dose-volume constraints for PTV and OARs

Gene	Sequence (5`-3`)	Primer lengt (bp)	Annealing temperature (℃)
β-actin	Forward CCTCGCCTTTGCCGATCC	102	60
	Reverse TTGCACATGCCGGAGCC	102	60
AKT	Forward TGGACGATAGCTTGGAGGGA	241	61
	Reverse ATTGTTCTGAGGGCTGAGGC	241	61
c-MET	Forward GAGAAGACTCCTACAACCCGAAT	287	59
	Reverse AGTACTAGCACTATGATGTCTCCC	287	59

Western blot analysis

Total protein was extracted from transplanted tumor tissue by means of a solution containing phosphatase inhibitors cocktail tablets, PMSF, and RIPA Lysis Buffer (100 mM), according to the manufacturer's protocol. The total amount of protein was determined with the BCA Kit. Western blot procedures were described above.

Statistical analysis

Quantitative data are shown as mean ± standard deviation. Two sets of data were analyzed using unpaired two-tailed Student's *t* test. One-way ANOVA was applied when homogeneity of variance assumptions were satisfied (multiple comparisons between groups were conducted using SNK analysis of variance); otherwise, the Kruskal-Wallis *H* test was used (multiple comparisons between groups were performed by Kruskal-Wallis analysis of variance). The data were analyzed using the SPSS software, version 19.0, and $P < 0.05$ was considered to indicate statistical significance. Probit regression analysis was used to calculate IC_{50} with a 95% confidence interval (CI).

Results

Effects of crizotinib on H1993, H2228, and A549 cells growth

The IC_{50} values of crizotinib for H1993 and H2228 cells were found to be 179 and 335 nM, respectively. A549 cells were nearly insensitive to crizotinib at concentrations below ~10 nM, and the IC_{50} value for A549 cells could not be determined even after crizotinib administration at a concentration of 10,000 nM (Fig. 1). Crizotinib inhibited the growth and proliferation of H1993 and H2228 cells in a concentration-dependent manner.

Apoptotic effects of crizotinib on H1993, H2228, and A549 cells

Crizotinib induced apoptosis of H1993 and H2228 cells, but not in A549 cells ($P < 0.05$). At 179 nM crizotinib,

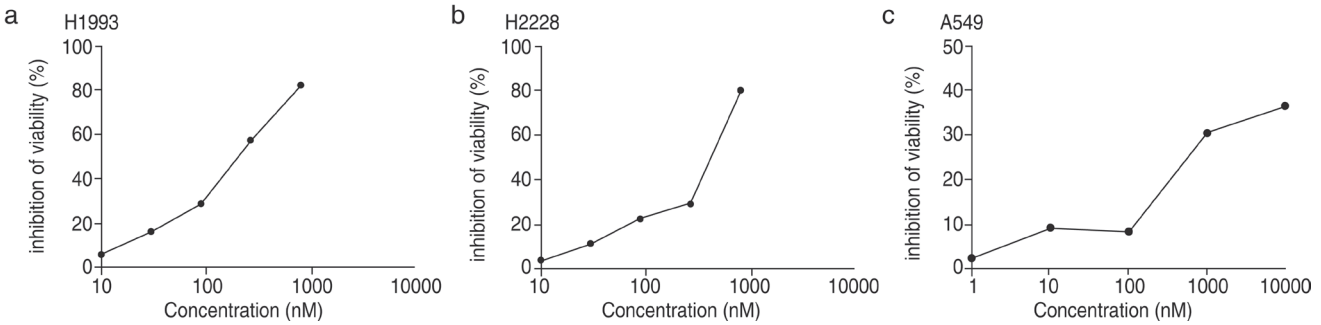


Fig. 1 Crizotinib inhibits the viability of 3 cell lines of NSCLC. H1993, H2228 and A549 cells were treated with crizotinib at different concentrations for 72 h. Percent inhibition was determined by the MTT assay. (a) The IC_{50} values of crizotinib for H1993 cells were found to be 179 nM; (b) The IC_{50} values of crizotinib for H2228 cells were found to be 335 nM; (c) A549 cells were nearly insensitive to crizotinib at concentrations below ~10 nM, and the IC_{50} value for A549 cells could not be determined even after crizotinib administration at a concentration of 10,000 nM.

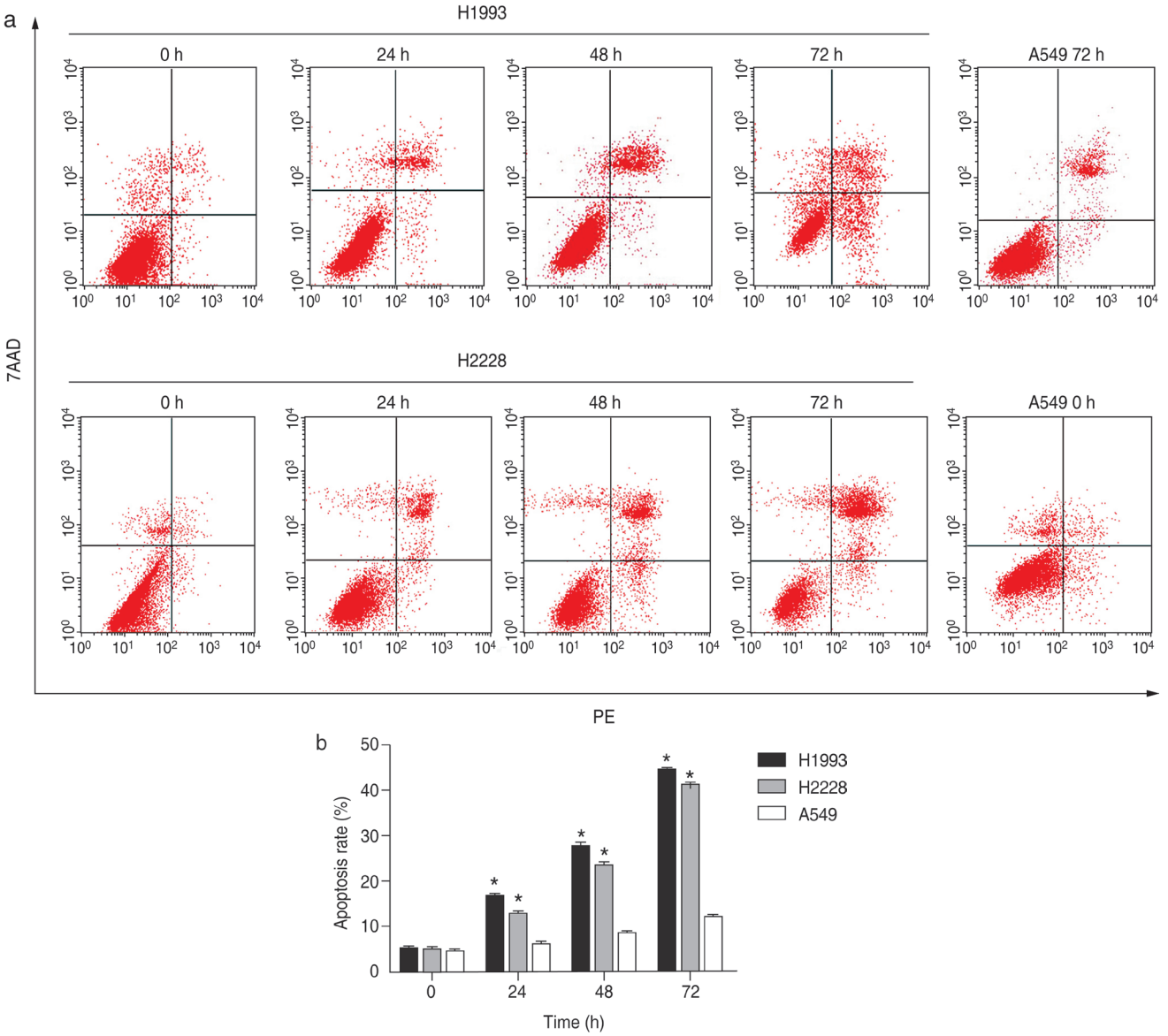


Fig. 2 Cell apoptosis rates of H1993, H2228 and A549 after treated with crizotinib at different times (0 h, 24 h, 48 h, 72 h). (a) Crizotinib induced apoptosis of H1993 and H2228 cells, but not in A549 cells ($P < 0.05$); (b) At 179 nM crizotinib, the apoptotic fractions of H1993 cells at 24, 48, and 72 h were 15.3% ± 2.1%, 27.2% ± 1.6%, and 46.5% ± 1.8%, respectively, while that of A549 cells at 72 h was 15.64% ± 0.61%. At 335 nM, the apoptotic fractions of H2228 cells at 24, 48, and 72 h were 13.7% ± 0.8%, 25.3% ± 1.6%, and 43.5% ± 3.2%, respectively.

the apoptotic fractions of H1993 cells at 24, 48, and 72 h were 15.3% ± 2.1%, 27.2% ± 1.6%, and 46.5% ± 1.8%, respectively, while that of A549 cells at 72 h was 15.64% ± 0.61%. At 335 nM, the apoptotic fractions of H2228 cells at 24, 48, and 72 h were 13.7% ± 0.8%, 25.3% ± 1.6%, and 43.5% ± 3.2%, respectively (Fig. 2). Crizotinib induced apoptosis of H1993 and H2228 cells in a time-dependent manner.

Effects of crizotinib on the expression of apoptosis-related proteins in the HGF/c-Met signaling pathway

The total amount of c-MET (c-MET plus p-c-MET) in H1993 cells treated with 200 nM crizotinib decreased as compared with untreated H1993 cells and with A549 cells. A significant decrease in the amounts of p-AKT, p-ERK, and p-c-MET was observed at 24 h after crizotinib treatment. Crizotinib decreased the amounts of p-AKT, p-ERK, and p-c-MET in H1993 cells in a time-dependent manner. The level of p-c-MET at 72 h was the lowest, as compared with that of p-AKT and p-ERK. In H2228 cells, expression levels of c-MET, AKT, and ERK after 300 nM crizotinib treatment were not detectable as compared with untreated cells. Nevertheless, significant decreases in the amounts of p-AKT, p-ERK, and p-c-MET were observed after 48 h. Minimal levels of those phospho-proteins were observed after 72 h. Amounts of c-MET, p-c-MET, AKT, p-AKT, ERK, and p-ERK in A549 cells showed no changes after treatment with 300 nM crizotinib (Fig. 3).

Viability of crizotinib-treated NSCLC nude mice

Average daily food intake and body weight before and after crizotinib administration in NSCLC nude mice were shown in Fig. 4. Compared with Group E, the decrease in daily food intake that occurred in the other groups was statistically significant ($P < 0.05$). The largest decrease was observed in Group A. There was no significant difference in daily food intake between Groups C and D ($P > 0.05$). As to body weight, increases were detected in Group B compared with Groups A, C, and D ($P < 0.001$). Only slow weight gains were observed in Groups A, C, and D

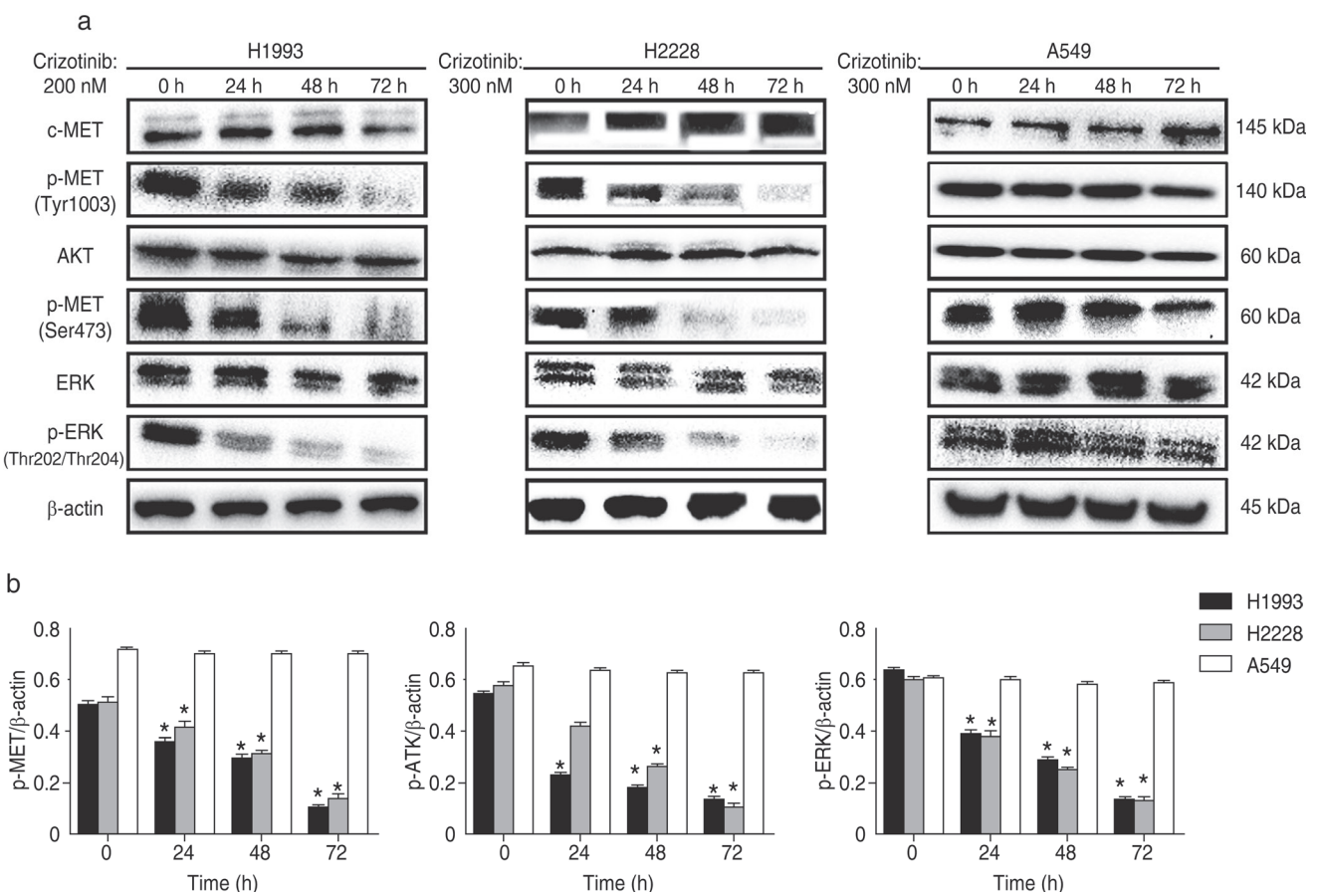


Fig. 3 Effect of crizotinib on expression of proteins in the 3 cell lines. (a) Protein levels in the 3 cell lines treated with crizotinib at different time-points (24 h, 48 h, 72 h) determined by Western blot; (b) Represents the relative expression of p-MET, p-AKT and p-ERK in 3 cells. n=3. *$P < 0.05$ compared with the control group (0 h).

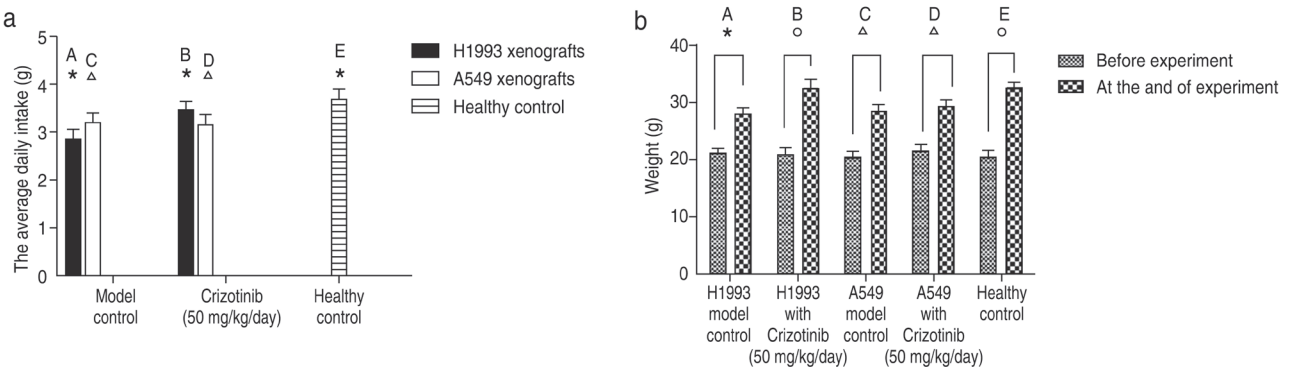


Fig. 4 Effects of crizotinib on life status of NSCLC xenograft tumor models. (a) Compared with other groups $^{*}P < 0.05$, compared with other groups except Group C or Group D $^{\triangle}P < 0.05$; (b) Compared with other groups $^{*}P < 0.001$, compared with other groups except Group C or Group D $^{\triangle}P < 0.001$, compared with other groups except Group B or Group E $^{\circ}P < 0.001$.

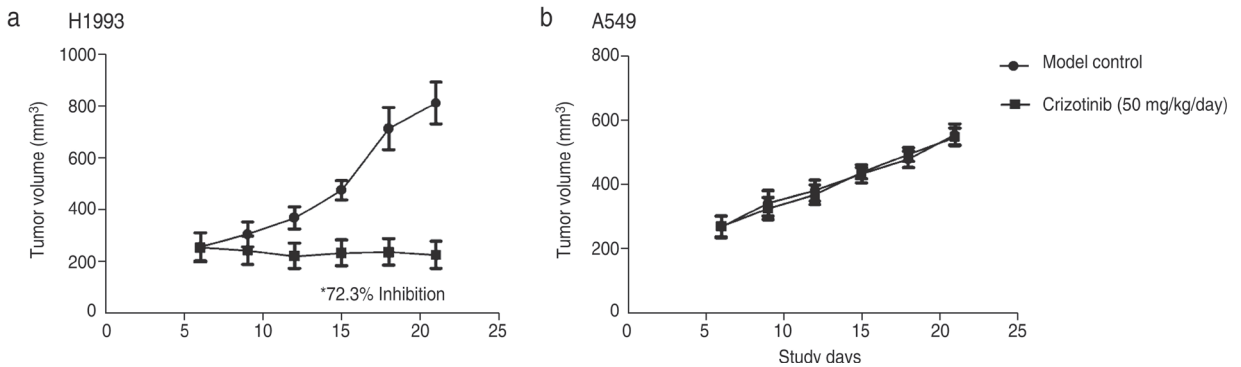


Fig. 5 NSCLC xenografts tumor size-time diagram. (a) The rate of inhibition of tumor volume for H1993 cells (in xenograft nude mice) by crizotinib was 72.3%, which is significantly higher than that in the control group ($P < 0.01$); (b) The inhibition of tumor volume of A549 cells in xenograft mice was only 1.3%, which is not different from that of the control group ($P > 0.05$).

compared with Group E ($P < 0.001$), with a remarkable difference for Group A. The results indicated that quality of life was better in the *c-Met* amplification NSCLC nude mice model and could be improved by treatment with crizotinib.

The tumor volume in NSCLC nude mice

The rate of inhibition of tumor volume for H1993 cells (in xenograft nude mice) by crizotinib was 72.3%, which is significantly higher than that in the control group ($P < 0.01$). The inhibition of tumor volume of A549 cells in xenograft mice was only 1.3%, which is not different from that of the control group ($P > 0.05$; Fig. 5). The results show that crizotinib exerts its growth inhibition on *c-Met* amplification-positive but not-negative NSCLC.

Amounts of HGF, c-MET, and p-c-MET in xenografts according to IHC analysis

The amounts of HGF, c-MET, and p-c-MET in 32 xenografts were determined by IHC analysis. The results showed that HGF is mainly located in the cytoplasm and the nucleus of tumor cells, and shows varying staining intensity. Positive expression of HGF in Groups A and B was higher than that of Groups C and D, but the differences were not statistically significant ($P > 0.05$). c-MET and p-c-MET were mainly located in the cytoplasm. Expression of c-MET in Groups A and B was also higher than that in Groups C and D, and the differences were statistically significant ($P < 0.0001$). The amount of p-c-MET in Group A was significantly higher than that in the other groups ($P < 0.0001$), while in group B, the amount of p-c-MET was the lowest. There were no differences in amounts of p-c-MET between Groups C and D (Fig. 6). The

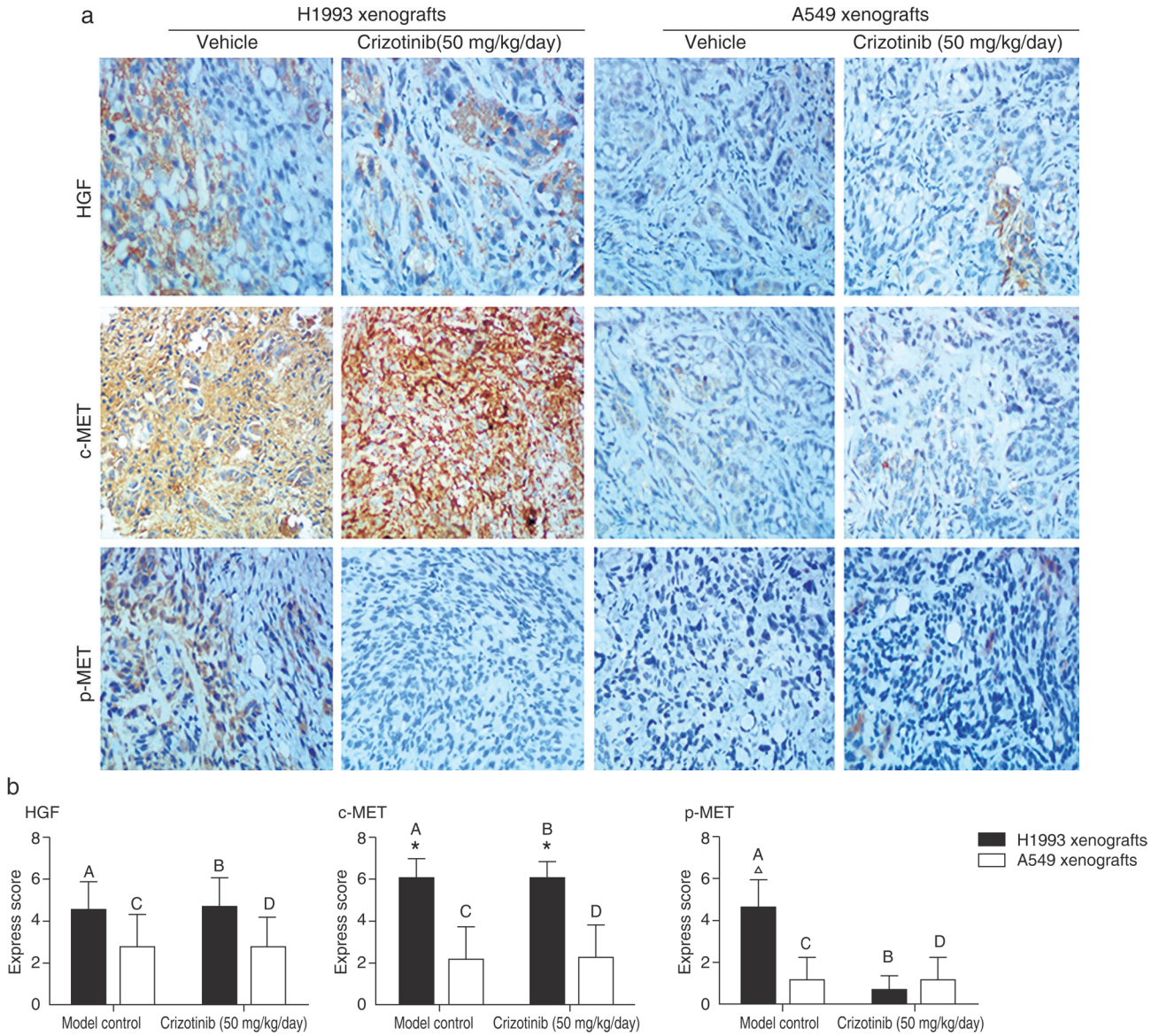


Fig. 6 Expression of HGF, c-MET, and p-MET in tumor tissues of NSCLC xenografts. (a) Original magnification×100; (b) n=8. Compared with other groups $^{\triangle}P < 0.0001$, compared with Group C and Group D $^{*}P < 0.0001$.

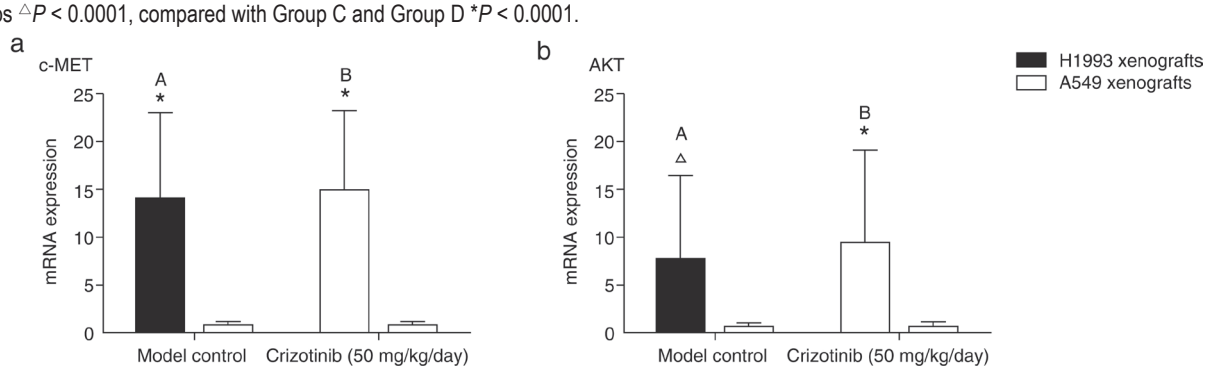


Fig. 7 Expression of c-MET, AKT mRNA in tumor tissues of NSCLC xenografts. (a)The mRNA expression of c-MET in Groups A and B were higher than that in Groups C and D, with statistical significance ($P < 0.001$, $P < 0.001$, respectively). In Group B showed no significant differences from Group A ($P > 0.05$). No differences between Groups C and D were detected ($P > 0.05$); (b)The mRNA expression of AKT in Groups A and B were higher than that in Groups C and D, with statistical significance ($P < 0.05$, $P < 0.001$, respectively). In Group B showed no significant differences from Group A ($P > 0.05$). No differences between Groups C and D were detected ($P > 0.05$).

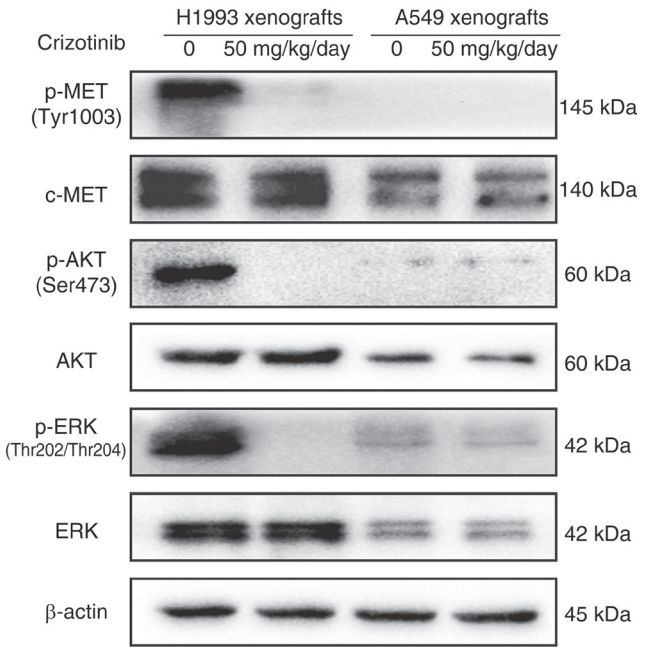


Fig. 8 Effect of crizotinib on expression of proteins in tumor tissues of NSCLC xenografts.

results show that the level of expression of HGF is unrelated to the status of *c-Met* amplification and the use of crizotinib. High expression of c-MET in H1993 cells can be inhibited by crizotinib, and this effect may contribute to the suppression of the growth of tumor tissue in nude mice harboring *c-Met* amplification in the xenograft.

Influence of crizotinib on mRNA expression of c-MET and AKT

The mRNA expression of c-MET and AKT in Groups A and B were higher than that in Groups C and D, with statistical significance ($P < 0.001$, $P < 0.001$, $P < 0.05$, and $P < 0.001$, respectively). The mRNA expression of c-MET and AKT in Group B showed no significant differences from Group A ($P > 0.05$). No differences between Groups C and D were detected ($P > 0.05$; Fig. 7). These results show that crizotinib had little or no effect on the mRNA expression of c-MET and AKT.

Influence of crizotinib on the expression of c-MET and related proteins of the Akt/Erk signaling pathway

Expression of c-MET, AKT, and ERK in xenografts was significantly higher in Groups A and B than in Groups C and D ($P < 0.001$, $P < 0.05$, and $P < 0.01$, respectively). Nonetheless, the difference between Groups A and B was not significant ($P > 0.05$). The amount of p-c-MET was higher in xenografts of Group A in comparison with the other groups, with statistical significance ($P < 0.05$). The expression of p-AKT in Group A was higher than that in the other groups ($P < 0.001$, $P < 0.05$, and $P < 0.05$, respectively). Compared with the other groups, the lowest expression of p-ERK was detected in Group B ($P < 0.01$; Fig. 8 and Table 2). The results indicate that crizotinib inhibits the growth of tumor tissue in H1993 xenografts by downregulating the phosphorylation of c-MET and related downstream proteins of the Akt/Erk signaling pathway.

Discussion

c-MET, which is encoded by the *c-Met* proto-oncogene, can be autophosphorylated on its intracellular tyrosine residues after binding its natural ligand HGF, and the phosphorylated protein subsequently activates a variety of key downstream signaling cascades, including the PI3K/Akt, Ras/MAPK, and survivin/STAT3 pathways [8]. Under normal conditions, activation of the HGF/c-Met pathway plays an essential regulatory role in embryonic development and tissue repair. In contrast, disturbances or overactivation of the HGF/c-Met pathway can promote cell proliferation, increase angiogenesis, enhance tumor invasion and metastasis, and decrease apoptosis [9].

Preclinical studies suggest that growth and survival of lung cancer cells harboring *c-Met* amplification depend on persistent activation of the HGF/c-Met pathway [5]. Clinically, secondary amplification of the *c-Met* may be involved in the development of EGFR-TKIs resistance [10]. Pao *et al* [11] proposed that cancer cells can activate the *c-Met* signaling pathway for sustained survival after blockade of the EGFR signaling pathway. The main cause of *c-Met* amplification is the breakage-fusion-bridge mechanism [12]. Based on the ratio of *c-Met* to centromere of chromosome 7, the degree of *c-Met* amplification is subdivided into 3 categories: 1.8–2.2 for

Table 2 Effects of crizotinib on indicated proteins expression in tumor tissues of NSCLC xenografts

Group	c-MET	p-MET	AKT	p-AKT	ERK	p-ERK
A	2.26 ± 0.45*	1.04 ± 0.30△	1.42 ± 0.36*	1.29 ± 0.30△	2.60 ± 0.90*	1.73 ± 1.58
B	2.24 ± 0.55*	0.25 ± 0.12	1.49 ± 0.41*	0.21 ± 0.10△	2.69 ± 0.85*	0.21 ± 0.09○
C	1.23 ± 0.28	0.36 ± 0.10	0.78 ± 0.16	0.55 ± 0.28	0.75 ± 0.21	0.77 ± 0.38
D	1.28 ± 0.31	0.35 ± 0.12	0.71 ± 0.17	0.68 ± 0.27	0.70 ± 0.20	0.82 ± 0.43

△, compared with each other groups: $P < 0.05$; *, compared with Group C and Group D : $P < 0.05$; ○, compared with other groups $P < 0.001$.

low amplification, 2.2–2.5 for moderate amplification, and ≥5 for a high degree of amplification. Commonly used in clinical trials involving a c-Met amplification-positive standard is a high *c-Met* gene copy number (mean ≥ 5 copies/cell) according to fluorescence *in situ* hybridization, as proposed by Cappuzzo in 2009 [13]. Amplification of *c-Met* leads to overexpression of receptor *c-MET*, which is susceptible to *c-MET* inhibitors. The development of crizotinib was originally aimed at *c-MET* (according to the eutectic structure of both PHA-665752 and the *c-MET* unphosphorylated kinase domain) via competitive inhibition of the binding and subsequent autophosphorylation of kinase *c-MET* and ATP hydrolysis.

Our findings also reveal the antiproliferative and proapoptotic effects of crizotinib on H1993 and H2228 cells. Inhibition by crizotinib is both time-dependent and concentration-dependent. Similar inhibition was not observed in A549 and crizotinib-untreated cells.

Akt, as a key molecule of the PI3K/Akt/mTOR signaling pathway, plays an important role by activating such molecules as mTOR and by phosphorylating such proteins as Bcl-2 and Fox, thus inhibiting apoptosis [14]. ERK is a downstream effector of the MAPK signaling pathway and can be activated by phosphorylation, and then p-ERK enters the nucleus to promote phosphorylation of transcription factor NF-κB and c-Myc and to enhance cell proliferation and to reverse proapoptotic effects of drugs [15]. The presence of p-AKT and p-ERK is viewed as the main sign of activation of the HGF/c-Met signaling pathway.

In this study, we evaluated the expression of c-MET, AKT, ERK, and their active forms of signaling proteins by western blotting and showed that the amounts of p-c-MET, p-AKT, and p-ERK in H1993 and H2228 cells are all decreased by crizotinib in a time-dependent manner. These results suggest that crizotinib inhibits activation of the Akt and Erk signaling pathways and downregulates the phosphorylation of AKT and ERK by decreasing c-MET activation (i.e., phosphorylation). These inhibitory effects promote apoptosis of tumor cells. This finding is consistent with the results of Junko and Akihiro [16,17].

In addition, we observed that complete disappearance of p-c-MET occurred at 72 h after crizotinib administration, whereas the expression of the downstream signaling proteins p-AKT and p-ERK was not suppressed completely. Thus, we assume that other pathways may be involved in activating the AKT and ERK downstream signaling pathways. It has been proven that c-MET inhibits apoptosis of lung cancer cells via activation of the p53 signaling pathway [18]. Belal *et al* [19] reported cross-talk between the c-Met and *EGFR* signaling pathways, which does not take place directly but is induced by Erk, c-Met, and other mediators. Blockage of the EGFR or Erk signaling pathways can decrease the expression and activation of c-MET in EGFR-mutant and wild-type NSCLC cells. In a phase I trial evaluating the safety and efficacy of crizotinib in advanced NSCLC with *c-Met* amplification (ClinicalTrials.gov NCT00585195), the effective rates of treatment in patients with low, moderate, and high *c-Met* amplification were 0%, 17%, 67%. A dose of crizotinib of 50 mg/kg of body weight daily was used in that trial and is similar to the dose used in the phase I clinical trial of crizotinib for the treatment of NSCLC patients with *ALK* fusion or *c-Met* amplification. In line with Tanizaki's results[16], our findings indicate that crizotinib significantly improves quality of life of the nude mice with *c-Met* amplification in the NSCLC xenograft. Food intake of the mice with a *c-Met* amplification-positive NSCLC xenograft was significantly decreased compared with that of the control groups. Of note, the weight loss that occurred in the *c-Met* amplification-positive model did not reach statistical significance, suggesting that known adverse effects of crizotinib such as nausea, vomiting, diarrhea, constipation, and other gastrointestinal symptoms were mild, and crizotinib was well tolerated. The general health condition of our mouse models of *c-Met* amplification-positive NSCLC was worse than that in the blank control group because of rapid proliferation of tumor cells, suggesting that growth and survival of tumor cells harboring *c-Met* amplification may depend on sustained activation of the HGF/c-Met signaling pathway [20].

In addition, crizotinib can effectively suppress the tumor growth of H1993 cell xenografts in nude mice by inhibiting tyrosine kinases of the HGF/c-Met pathway. Similar results were not observed in the A549 cell xenograft models. Zou *et al* [21] found that crizotinib exerts its inhibition on c-MET tyrosine kinase activity in animal models of xenografts with *c-Met* amplification (gastric cancer, NSCLC, kidney, and prostate cancer cells), thus inducing apoptosis and attenuating ERK phosphorylation of the downstream AKT and MAPK pathways; this effect is similar to inhibition of the tyrosine kinase of *ALK* fusion variants under the influence of crizotinib. Our findings also show that crizotinib decreases the amounts of p-AKT and p-ERK by inhibiting c-MET phosphorylation instead of inhibiting expression of AKT and ERK during its *in vivo* antitumor action.

Other related studies show that strong phosphorylation of c-MET detected in H1993 cells may not be caused by activation by its ligand HGF. The c-Met knockdown by means of short hairpin RNA (shRNA) results in significant morphological changes of H1993 cells, whereas the proliferation of A549 cells is not inhibited by c-Met shRNA [22]. Our findings reveal that expression of HGF is not strongly related to *c-Met* status and crizotinib use. High expression of c-MET in c-Met amplification-

positive lung adenocarcinoma cells also indicates that activation of the c-Met signaling pathway is necessary for the survival of NSCLC.

It should be noted that in this study, we chose cell lines without *ALK* rearrangements for pharmacological experiments with c-MET. Nonetheless, owing to the expression of wild-type *ALK* gene in the selected cells, the pharmacological activities of crizotinib via ALK cannot be ruled out. Patients with advanced NSCLC with concomitant *EGFR* mutation and c-MET overexpression have significantly lower response rates to EGFR-TKIs, indicating that c-MET overexpression may cause intrinsic resistance to EGFR-TKIs. A combination of EGFR-TKIs and a c-MET inhibitor is a good strategy to overcome acquired resistance in patients with a c-MET–overexpressing tumor. One of the possible mechanisms of resistance may contribute to activities of both c-Met and EGFR signaling pathways.

Conclusion

This study shows that crizotinib, as a potent ATP-competitive inhibitor targeting kinase c-MET, can effectively inhibit the growth of H1993 and H2228 cells and the corresponding xenografts in nude mice. The inhibitory effect is directly related to the inhibition of c-MET phosphorylation and protein phosphorylation in its downstream pathway. Our findings provide the experimental basis and clinical reference for the use of c-MET inhibitors in the treatment of patients with NSCLC harboring *c-Met* amplification.

Conflicts of interest

The authors have no potential conflicts of interest.

References

1. Gherardi E, Birchmeier W, Birchmeier C, *et al*. Targeting MET in cancer: rationale and progress. Nat Rev Cancer, 2012, 12: 89–103.
2. Gibney GT, Aziz SA, Camp RL, *et al*. c-Met is a prognostic marker and potential therapeutic target in clear cell renal cell carcinoma. Ann Oncol, 2013, 24: 343–349.
3. Trovato M, Torre ML, Ragonese M, *et al*. HGF/c-met system targeting PI3K/AKT and STAT3/phosphorylated-STAT3 pathways in pituitary adenomas: an immunohistochemical characterization in view of targeted therapies. Endocrine, 2013, 44: 735–743.
4. Comprehensive molecular profiling of lung adenocarcinoma. Nature, 2014, 511: 543–550.
5. Lutterbach B, Zeng Q, Davis LJ, *et al*. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res, 2007, 67: 2081–2088.
6. Paik PK, Drilon A, Fan PD, *et al*. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov, 2015, 5: 842–849.
7. Caparica R, Yen CT, Coudry R, *et al*. Responses to crizotinib can occur in high-level MET-amplified non-small cell lung cancer independent of MET exon 14 alterations. J Thorac Oncol, 2017, 12: 141–144.
8. Gandhi L, Janne PA. Crizotinib for ALK-rearranged non-small cell lung cancer: a new targeted therapy for a new target. Clin Cancer Res, 2012, 18: 3737–3742.
9. Klapper LN, Kirschbaum MH, Sela M, *et al*. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. Adv Cancer Res, 2000, 77: 25–79.
10. Engelman JA, Zejnullahu K, Mitsudomi T, *et al*. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science, 2007, 316: 1039–1043.
11. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer, 2010, 10: 760–774.
12. Smolen GA, Sordella R, Muir B, *et al*. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci USA, 2006, 103: 2316–2321.
13. Cappuzzo F, Marchetti A, Skokan M, *et al*. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol, 2009, 27: 1667–1674.
14. Zimmer S, Kahl P, Buhl TM, *et al*. Epidermal growth factor receptor mutations in non-small cell lung cancer influence downstream Akt, MAPK and Stat3 signaling. J Cancer Res Clin Oncol, 2009, 135: 723–730.
15. Ehrenfeld P, Conejeros I, Pavicic MF, *et al*. Activation of kinin B1 receptor increases the release of metalloproteases-2 and -9 from both estrogen-sensitive and - insensitive breast cancer cells. Cancer Lett, 2011, 301: 106–118.
16. Tanizaki J, Okamoto I, Okamoto K, *et al*. MET tyrosine kinase inhibitor crizotinib (PF-02341066) shows differential antitumor effects in non-small cell lung cancer according to MET alterations. J Thorac Oncol, 2011, 6: 1624–1631.
17. Kogita A, Togashi Y, Hayashi H, *et al*. Activated MET acts as a salvage signal after treatment with alectinib, a selective ALK inhibitor, in ALK-positive non-small cell lung cancer. Int J Oncol, 2015, 46: 1025–1030.
18. Liu Y, Liu JH, Chai K, et al. Inhibition of c-Met promoted apoptosis, autophagy and loss of the mitochondrial transmembrane potential in oridonin-induced A549 lung cancer cells. J Pharm Pharmacol, 2013,65: 1622–1642.
19. Belalcazar A, Azana D, Perez CA, *et al*. Targeting the Met pathway in lung cancer. Expert Rev Anticancer Ther, 2012, 12: 519–528.
20. Timofeevski SL, McTigue MA, Ryan K, *et al*. Enzymatic characterization of c-Met receptor tyrosine kinase oncogenic mutants and kinetic studies with aminopyridine and triazolopyrazine inhibitors. Biochemistry, 2009, 48: 5339–5349.
21. Zou HY, Li Q, Lee JH, *et al*. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. Cancer Res, 2007, 67: 4408–4417.
22. Lutterbach B, Zeng Q, Davis LJ, *et al*. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res, 2007, 67: 2081–2088.

DOI 10.1007/s10330-016-0210-0
Cite this article as: Zhou SZ, Dong ZX, Lv JY, *et al*. The role of the HGF/c-Met signaling pathway in crizotinib-induced apoptosis in lung cancer with c-Met amplification. Oncol Transl Med, 2017, 3: 116–126.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0217-7

June 2017, Vol. 3, No. 3, P127–P130

ORIGINAL ARTICLE

The expression of estrogen receptors in thyroid cancer and its significance

Yuxuan Che[1], Huamin Qin[2] (Co-first author), Xiaolei Ding[1] (✉), Xiuhua Sun[1], Lifen Wang[2]

[1] Medical Oncology Department, The Second Affiliated Hospital, Dalian Medical University, Dalian 116027, China
[2] Pathology Department, The Second Affiliated Hospital, Dalian Medical University, Dalian 116027, China

Abstract

Objective The study aimed to detect the expression of estrogen receptors (ERs) in thyroid cancer and investigate the correlation between their expression and clinical features and different pathological types.

Methods The expression of ERs in 56 samples of thyroid cancer tissues was detected by an immunochemical approach. The expression of ERs in thyroid cancer tissues and different pathological types were analyzed using the χ^2 test.

Results The number of cases with positive expression of ER in thyroid cancer tissues was 36. The number of papillary thyroid cancers (PTCs) was 48, with positive expression of ERs in 32 cases. The number of follicular thyroid cancers was 4, with positive expression of ERs in 2 cases. The number of medullary thyroid cancers was 4, with negative expression of ERs in all cases. The difference between the expression and different pathological types showed statistical significance. The expression of ERs showed no correlation with sex, age, or TNM stage, with no statistical significance. However, the expression of ERs was correlated with metastasis of lymph nodes, which had statistical significance. The expression of ERs was negatively correlated with pathological types and metastasis of lymph nodes. The correlated coefficient index was –0.313 and –0.334, respectively.

Conclusion The expression of ERs showed no correlation with sex, age, or TNM stage, but was negatively correlated with pathological types and metastasis of lymph nodes.

Received: 28 December 2016
Revised: 14 March 2017
Accepted: 5 May 2017

Key words: thyroid cancer; estrogen receptor (ER); pathological type

Although the incidence of thyroid cancer accounts for 5% of thyroid nodules [1] and 1% of all tumors, thyroid cancer is the most common cancer of the endocrine glands [2]. According to the data from the American Cancer Society, the incidence of thyroid cancer has increased significantly since the 1990s. Some reports demonstrated that the incidence of thyroid cancer shows no gender difference before the age of 10. However, the female incidence was three or four times higher than the male incidence after 10 years of age and women of child-bearing age were the major proportion of female patients, although the incidence decreased in postmenopausal women [3–4]. Sungwalee *et al* proposed that women with early menarche, oral contraceptive use, or no pregnancy tend to suffer from thyroid cancer to some extent [5]. Thus, it can be seen that endogenous estrogen does play an important role in the occurrence and development of thyroid cancer.

Materials and methods

Tissues and clinical data were obtained from 56 thyroid cancer patients who underwent radical thyroidectomy in the Second Affiliated Hospital of Dalian Medical University (China) from October 2014 to September 2015 and the pathological diagnosis was confirmed as thyroid cancer. All 56 patients had not accepted chemotherapy or radiotherapy before the operation. The number of male patients was 19 and that of female patients was 37. Their ages ranged from 23- to 71-years-old and the average age was (63.20 ± 10.18) years. There were 32 patients under 45-years-old and 24 patients beyond 45-years-old. Regarding the TNM stage, there were 31 patients with T1 and T2 stages, and 25 with T3 and T4 stages. There were 24 patients with lymph node metastasis and 32 patients without lymph node metastasis. Regarding the pathological

✉ Correspondence to: Xiaolei Ding. Email: 3038668@vip.sina.com.

type, there were 48 patients with papillary thyroid cancer (PTC), 4 patients with medullary thyroid cancer, and 4 patients with follicular thyroid cancer.

The expression of estrogen receptors (ERs) in the 56 tissue samples of thyroid cancer was detected by an immunochemical approach. The expression of ERs in thyroid cancer tissues and different pathologic types were analyzed by the χ^2 test. The value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The expression of estrogen receptors in thyroid cancer tissues

The positive expression result of ERs in thyroid cancer tissues was showed in Table 1. The positive expression of ERs displayed brownish yellow granules which mainly locates on the cell nucleus, partially on the cytoplasm (Fig. 1–4).

The relationship of estrogen receptors expression in thyroid cancer tissues and clinical features

The relationship of ERs expression in thyroid cancer tissues and clinical features was showed in Table 2. The expression of ERs has no correlation with sex, age and TNM stage, which has no statistical difference ($P > 0.05$). There are 24 patients with lymph metastasis and 32 patients without lymph metastasis, which had statistical significance ($P = 0.013$).

The expression of estrogen receptors in the different histopathological types

The expression result of ERs in the different histopathological types was shown in Table 3, and the expression had statistical significance ($P = 0.029$).

Table 1 The expression of estrogen receptors in thyroid cancer tissues

Group	Cases	Positive cases	Positive rate (%)
Thyroid cancer tissues	56	36	64.3

Table 2 The relationship of estrogen receptors expression in thyroid cancer tissues and clinical features

Clinical features	Cases	Estrogen receptors			χ^2	P
		Positive cases	Negative cases	Positive rate (%)		
Sex					0.016	0.900
Male	19	12	7	63.2		
Female	37	24	13	64.9		
Age (years)					0.104	0.747
≤ 45	32	20	12	66.7		
> 45	24	16	8	62.5		
Stage					1.350	0.245
T1 + T2	31	22	9	71.0		
T3 + T4	25	14	11	56.0		
Lymph metastasis					6.229	0.013
Yes	24	11	13	45.8		
No	32	25	7	78.1		

Table 3 The expression of estrogen receptors in the different histopathological types

Groups	Cases	Positive cases	Positive rate (%)	χ^2	P
Papollary carcinoma	48	32	66.7	7.087	0.029
Follicular carcinoma	4	2	50.0		
Medullary carcinoma	4	0	0.0		

Discussion

Thyroid cancer is the most common malignancy of the endocrine system [2] with increasing incidence, especially of papillary carcinoma. Eheman *et al* demonstrated that the male incidence of thyroid cancer was 5.5/100 000 and the female incidence was 16.3/100 000 from 2004 to 2008 in America [6]. The occurrence of thyroid cancer may be related to the exposure to radiation or hyperplastic diseases of thyroid tissues in family history [7–9]. Moreover, an increasing number of studies show that endogenous estrogen may play an important role in the occurrence and progression of thyroid cancer.

ERs play an important role in non-genomic or classical genomic estrogen signaling. Estrogen enters target cells via passive diffusion, and then the binding of ligand E2 to ERs results in the conformational changes of ERs. These conformational changes cause the dissociation of the ER from its ligand proteins, and then regulate gene expression through the combination of homo- or hetero-dimerization of E2-ER and the nucleotide sequence located in the promoters of target genes, known as estrogen response elements (EREs). However, in humans, one-third of genes regulated by estrogen do not contain ERE-sequences [10–11]. Under these circumstances, estrogen can regulate the expression of target genes by modulating the functions of other transcriptional factors in the nucleus, which results in chromatin alteration via the interactions between proteins. This process is collectively known as the ERE-independent genomic actions of estrogen [12]. Estrogen exerts biological effects in the bone, breast, vasculature, and nervous system through its interaction with related estrogen binding proteins on the cell membrane, instead of the gene transcription and protein synthesis mediated by E2-ER. These actions are referred to as non-genomic estrogen signaling.

Estrogen signaling is mediated primarily through two isoforms of the ER: ERα and ERβ. ERα can promote the growth of thyroid cancer cells (including papillary

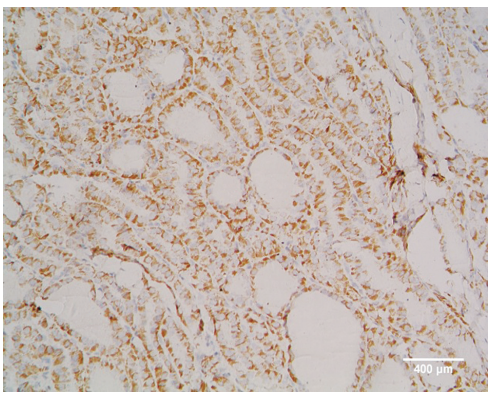


Fig. 1 The positive expression of estrogen receptors in papillary thyroid cancer cells (HE, ×200)

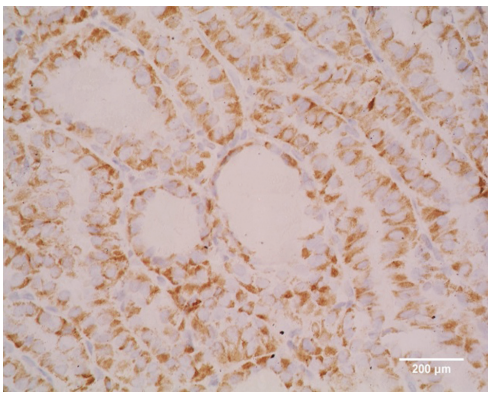


Fig. 2 The positive expression of estrogen receptors in papillary thyroid cancer cells (HE, ×400)

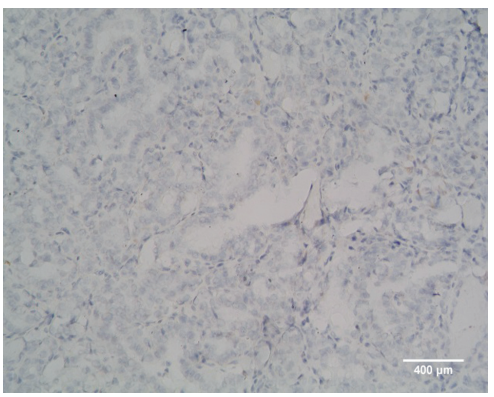


Fig. 3 The negative expression of estrogen receptors in thyroid thyroid cancer cells (HE, ×200)

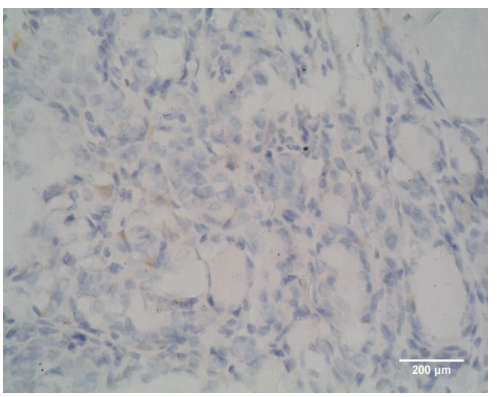


Fig. 4 The negative expression of estrogen receptors in thyroid cancer cells (HE, ×400)

cancer cells KAT5, follicular cancer cells FRO, and undifferentiated cancer cells ARO) by integrating with E2 [13]. Zeng *et al* demonstrated that the agonist and antagonist for ERα and ERβ can regulate the expression of ERs. Propylpyrazole triol (PPT), an ERα agonist, can enhance the proliferation of thyroid cancer cells and the expression of antiapoptotic protein Bcl-2, while diarylpropionitrile (DPN), an ERβ agonist, can inhibit the proliferation and enhance the expression of apoptotic gene Bax. In addition, the knockdown of ERα can significantly reduce the expression of Bcl-2, while the silencing of ERβ can enhance the expression of Bcl-2; this expression imbalance between ERα and ERβ may result in the occurrence of thyroid cancer [13]. ERα can promote the growth of cancer cells, while ERβ can inhibit the proliferation of cancer cells. Some reports proposed that the increased ratio of ERα to ERβ might influence the growth and progression of medullary thyroid cancer [14]. By investigating the genotype of 344 PTCs and 452 controls, Schonfeld *et al* identified that seven single nucleotide polymorphisms (SNPs) may relate to the development of PTC, four of which are located on the *CYP19A1*, *ESR1*, *HSD17B3*, and *SULF1* genes. However, they demonstrated that all the SNPs had no obvious correlation with the occurrence and progression of PTC [15]. On the other hand, RebaÏ *et al* reported that the polymorphism change in the A549G codon on the ER ESR1 might alter the recognition and combination of ER ligands, which may result in the occurrence of thyroid cancer [16]. Rajoria *et al* also identified that the expression of ERα and ERβ were both detected in the PTC cell lines KAT5, NPA87, and BCPAP, and E2 could promote the adhesion, invasion, and metastasis of BCPAP cells [17–18]. Dong *et al* found that E2 exposure can lead to the downregulation of E-cadherin and upregulation of vimentin and matrix metalloproteinase-9 (MMP-9). Additionally, ERα and ERβ play significant roles in BCPAP cell metastasis through the differential regulation of E-cadherin, vimentin, and MMP-9 [19]. In our study, we found that the positive expression of ERs was different in the three types of thyroid cancers, which indicated that the higher the expression of ER, the higher the

differentiation of thyroid cancer. In addition, we also found that the positive expression of ERs could indicate thyroid cancer without the metastasis of lymph nodes.

Conclusion

As a whole, our data demonstrates that the expression of ERs may correlate with the degree of differentiation and the metastasis of lymph nodes, and it might be a prognostic indicator that can be used in targeting ERs for the treatment of thyroid cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Mackenzie EJ, Mortimer RH. Thyroid nodule and thyroid cancer. Med J Aust, 2004, 180: 242–247.
2. Nix P, Nicolaides A, Coatesworth AP. Thyroid cancer review 1: presentation and investigation of thyroid cancer. Int J Clin Pract, 2005, 59: 1340–1344.
3. Libutti SK. Understanding the role of gender in the incidence of thyroid cancer. Cancer J, 2005, 11: 104–105.
4. Chen GG, Vlantis AC, Zeng Q, *et al*. Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. Curr Cancer Drug Targets, 2008, 8: 367–377.
5. Sungwalee W, Vatanasapt P, Kamsa-Ard S, *et al*. Reproductive risk factors for thyroid cancer: a prospective cohort study in Khon Kaen, Thailand. Asian Pac J Cancer Prev, 2013, 14: 5153–5155.
6. Eheman C, Henley SJ, Ballard-Barbash R, *et al.* Annual report to the Nation on the status of cancer, 1975–2008, featuring cancers associated with excess weight and lack of sufficient physical activity. Cancer, 2012, 118: 2338–2366.
7. Franceschi S, Preston-Martin S, Dal Maso L, *et al*. A pooled analysis of case-control studies of thyroid cancer. IV. Benign thyroid diseases. Cancer Causes Control, 1999, 10: 583–595.
8. Horn-Ross PL, Morris JS, Lee M, *et al*. Iodine and thyroid cancer risk among women in a multiethnic population: the Bay Area Thyroid Cancer Study. Cancer Epidemiol Biomark Prev, 2001, 10: 979–985.
9. Preston-Martin S, Franceschi S, Ron E, *et al*. Thyroid cancer pooled analysis from 14 case-control studies: what have we learned? Cancer Causes Control, 2003, 14: 787–789.
10. Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol, 2005, 19: 833–842.
11. O'Lone R, Frith MC, Karlsson EK, *et al*. Genomic targets of nuclear estrogen receptors. Mol Endocrinol, 2004, 18: 1859–1875.
12. Vanacker JM, Pettersson K, Gustafsson JA, *et al*. Transcriptional targets shared by estrogen receptor-related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. EMBO J, 1999, 18: 4270–4279.
13. Zeng Q, Chen GG, Vlantis AC, *et al*. Oestrogen mediates the growth of human thyroid carcinoma cells via an oestrogen receptor-ERK pathway. Cell Prolif, 2007, 40: 921–935.
14. Cho MA, Lee MK, Nam KH, *et al.* Expression and role of estrogen receptor alpha and beta in medullary thyroid carcinoma: different roles in cancer growth and apoptosis. J Endocrinol, 2007, 195: 255–263.
15. Schonfeld SJ, Neta G, Sturgis EM, *et al.* Common genetic variants in sex hormone pathway genes and papillary thyroid cancer risk. Thyroid, 2012, 2: 151–156.
16. Rebaï M, Kallel I, Charfeddine S, *et al*. Association of polymorphisms in estrogen and thyroid hormone receptors with thyroid cancer risk. J Recept Signal Transduct Res, 2009, 29: 113–118.
17. Rajoria S, Suriano R, Shanmugam A, *et al*. Metastatic phenotype is regulated by estrogen in thyroid cells. Thyroid, 2010, 20: 33–41.
18. Kumar A, Klinge CM, Goldstein RE. Estradiol-induced proliferation of papillary and follicular thyroid cancer cells is mediated by estrogen receptors alpha and beta. Int J Oncol, 2010, 36: 1067–1080.
19. Dong W, Zhang H, Li J, *et al*. Estrogen induces metastatic potential of papillary thyroid cancer cells through estrogen receptor α and β. Int J Endocrinol, 2013, 941568. Epub 2013 Oct 10.

DOI 10.1007/s10330-016-0217-7
Cite this article as: Che YX, Qin HM, Ding XL, *et al*. The expression of estrogen receptors in thyroid cancer and its significance. Oncol Transl Med, 2017, 3: 127–130.

Oncology and Translational Medicine
DOI 10.1007/s10330-016-0201-1
2017, Vol. 3, No. 3, P131–P135

REVIEW ARTICLE

Roles of endoplasmic reticulum stress and apoptosis signaling pathways in gynecologic tumor cells: A systematic review

Kangsheng Liu[1], Weimin Fang[1], Erhu Sun[2], Yajun Chen[1] (✉)

[1] Department of Clinical Laboratory, Nanjing Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing 210029, China
[2] Department of Obstetrics and Gynecology, Nanjing Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing 210029, China

Abstract

Efficient functioning of the endoplasmic reticulum (ER) is very important for most cellular activities, such as protein folding and modification. The ER closely interacts with other organelles, including the Golgi body, endosome, membrane, and mitochondria, providing lipids and proteins for the repair of these organelles. ER stress can be induced by various abnormal materials in the cell. ER stress is a compensatory intracellular environment disorder that occurs during areaction. ER can sense the stress and respond to it through translational attenuation, upregulation of the genes for ER chaperones and related proteins, and degradation of unfolded proteins by a quality-control system, but excessive ER activation can cause cell death. The Pubmed and Web of Science databases were searched for full-text articles, and the terms "endoplasmic reticulum stress / unfolded protein response / gynecologic tumor cell apoptosis" were used as key words. Thirty-five studies of ER stress and unfolded protein response published from 2000 to 2016 were analyzed. Stress triggers apoptosis through a variety of signaling pathways. Increasing evidence has shown that the ER plays an important role in tumor cell diseases. The present review discusses the molecular mechanisms underlying unfolded protein response and its ability to promote survival and proliferation in gynecologic tumor cells.

Key words: endoplasmic reticulum (ER); unfolded protein response (UPR); inositol-requiring-JNK (IRE1-JNK); caspase; CCAAT-enhancer-binding protein homologous protein (CHOP); gynecologic tumor cell

List of abbreviations: IRE1α: inositol-requiring enzyme 1α; UPR: unfolded protein response; XBP-1: X binding protein; PERK: protein kinase RNA-like ER kinase; GRP78: glucose-regulated protein 78; Tun: tunicamycin; CHOP: CCAAT-enhancer-binding protein homologous protein; ERSE: endoplasmic reticulum stress element; ASK1: apoptosis signal-regulating kinase 1; eIF-2alpha: eukaryotic translation initiation factor 2; IP3: inositol 1,4,5-trisphosphat; ERO1: endoplasmic reticulum oxidoreductin 1; TMEM214: transmembrane protein 214; GADD34: cofactor of eIF2.phosphatase; H1299: human hung carcinoma H1299 cells; SH-SY5Y: human neuroblastoma cells; JNK: c-Jun N-terminal kinase; VEGFA: vascular endothelial growth factor-A; SERCA: sarcoplasmic reticulum Ca^{2+}-ATPase; NAFLD: nonalcoholic fatty liver disease; ATF4: activating transcription factor 4; Pim-1: proviral integration moloney virus; PUMA: p53-upregulated modulator of apoptosis

Received: 23 October 2016
Revised: 19 December 2016
Accepted: 10 January 2017

The endoplasmic reticulum (ER) is the largest membrane-enclosed organelle in cells and is responsible for the synthesis, processing, and modification of proteins [1]. Conditions that interfere with ER function, such as virus infection and calcium homeostasis, lead to the accumulation and aggregation of unfold proteins, causing severe ER stress. The subsequent unfolded protein response (UPR) is a cellular attempt to lower the burden on the ER and restore ER homeostasis by imposing a general arrest in protein synthesis, upregulating chaperone proteins,

✉ Correspondence to: Yajun Chen. Email: fycheyajun201601@163.com

and degrading misfolded proteins. An increasing number of studies has shown that persistent ER stress may largely result from an altered state of the UPR [2]. ER stress response / UPR signaling pathways are activated in primary solid tumors as a result of cell-intrinsic defects, such as dysregulation of protein synthesis, folding, and secretion, and also as a consequence of microenvironment changes. This article mainly describes the research progress of the gynecologic tumor cell apoptosis pathway and ER stress, which has provided important insights into the molecular mechanisms of ER stress-induced apoptosis.

ER stress signaling pathways

IRE1 signaling pathways

IRE1, which is activated in response to the accumulation of unfolded proteins, determines the splicing of a 26-nucleotide-long intron of mRNA encoding the transcription factor X box binding protein 1 (XBP1). The generated splicing variant, XBP1, acts as a transcription factor that moves to the nucleus and causes the transcription of genes coding ER chaperones in order to mitigate the stress [3-4]. Both IRE1 molecules respond to the accumulation of unfolded proteins in the ER, which activate their kinase and thus their RNase activities [5-6]. IRE1 and PERK are two type I transmembrane ER-localized protein kinase receptors that signal the UPR through a process involving homodimerization and autophosphorylation [7].

The IRE1-JNK pathway is an important signaling pathway that helps cells survive via the UPR. First, the endoribonuclease activity of IRE1 cleaves XBP-1 mRNA, converting it into a potent transcriptional activator that, in turn, induces the gene expression of proteins involved in protein degradation [8–9]. Second, recent studies have shown that IRE1 links ER stress to the activation of JNK signaling pathways. Specifically, IRE1 binds to TRAF2 and through its kinase activity couples ER stress to the activation of JNK [10]. The activation of JNK by ER stress requires the presence of ASK1 [11]. IRE1 recruitment of protein kinase adapter TRAF2 (tumor) can be caused by permanent ER stress, which activates ASK and JNK protein kinase and initiates the apoptotic cascade [12]. The IRE1/XBP-1 pathway is required for efficient protein folding, maturation, and degradation in the ER and suggests the existence of subsets of UPR target genes, as defined by their dependence on XBP-1. ASK1 oligomerization promotes conformational changes induced by an IRE1-TRAF2 interaction, which also promotes Thr845 intermolecular phosphorylation in the ASK1 activation cycle (IRE-TRAF2-ASK1).

PERK signaling pathway

Oligomerization and ER stress increase PERK protein-kinase activity; PERK phosphorylates eIF2 on serine residue 51, inhibiting the translation of messenger RNA into protein. On one hand, protein translation and synthesis can be inhibited by phosphorylation of eIF2a to relieve ER pressure. On the other hand, phosphorylation of eIF2a can selectively activate the translation of ATF4, increase the synthesis of its binding partner, and affect amino acid metabolism. Inhibiting eIF2-GDP in the eIF2-GTP energy cycle can inhibit translation and reduce protein synthesis [13]. A previous study showed that activation and phosphorylation of PERK selectively enhance its affinity for the non-phosphorylated eIF2 complex. This switch is correlated with a significant change in the protease sensitivity pattern, indicating a major conformational change in the PERK kinase domain upon activation. Although it is dispensable for catalytic activity, PERK's kinase insert loop is required for substrate binding and eIF2alpha phosphorylation *in vivo*. A previous study revealed a novel mechanism for eIF2 recruitment by activated PERK and for unidirectional substrate flow in the phosphorylation reaction [14].

Previous studies showed that CHOP directly activates GADD34, which promotes ER client protein biosynthesis. Thus, impaired GADD34 expression reduces client protein load. Furthermore, mice lacking GADD34-directed eIF2alpha dephosphorylation (such as $CHOP^{-/-}$ mice) are resistant to renal toxicity of the ER stress-inducing drug tunicamycin. CHOP (promoter regions have four cis-response elements) can induce the expression of apoptosis proteins such as GADD34, ERO1, and DR5. ER transmembrane receptors detect the onset of ER stress and initiate the UPR to restore normal ER function. Recent studies have shown that prolonged stress or failures in the adaptive response are likely mediated by increases in target proteins, such as oxidase ERO1a (ER lumen protein), which transfer selectrons to molecular oxygen during disulfide bond formation [15]. Therefore, excessive expression of CHOP can lead to the generation of reactive oxygen species (ROS), ER stress, and apoptosis. In general, CHOP-GADD34-ERO1 partially mediates the ER stress signaling pathways that regulate cell apoptosis [16].

Caspase signaling pathway

It is generally thought that caspase-12, an ER outer membrane protein, is a specific apoptosis molecule involved in ER stress. Murine caspase-12 has an ER-associated proximal effect or activates procaspase-9 to cleave procaspase-3, leading directly to apoptosis [17]. However, human caspase-12 has no similar function because its gene is disrupted by a frame shift, resulting in a premature stop codon. In addition, human caspase-12

also contains amino acid substitutions in sites critical for caspase activity [18]. In contrast, human caspase-4 is specifically cleaved under ER stress, suggesting that it may be a functional ortholog of mouse caspase-12 in ER stress-induced apoptosis [19].

Recent studies have shown that caspase-4 participates in reactions in the human neuronal cell line SH-SY5Y [20]. Additionally, caspase-4 plays an important role as an innate immune effect or for discriminating between pathogenic and nonpathogenic bacteria [21]. Yamamuro showed that caspase-4 directly activates caspase-9 [22]. Reddy found that in contrast to the UPR, GRP78 overexpression does not result in G1 arrest or depletion of topoisomerase [23].

Some previous studies showed that a subpopulation of GRP78 can exist as an ER transmembrane protein as well as co-localize with caspase-7. A GRP78 mutant with no ATP binding domain failed to bind procaspase-7 and lost its protective effect against etoposide-induced apoptosis [23]. Li identified a critical mediator of ER stress-induced apoptosis, human transmembrane protein 214 (TMEM214) [24]. Over expression of TMEM214 induced apoptosis, whereas knockdown of TMEM214 inhibited ER stress-induced apoptosis, suggesting that TMEM214 is essential for ER stress-induced apoptosis by acting as an anchor for recruitment of procaspase-4 to the ER and its subsequent activation [24].

ER stress in gynecologic tumor cells

GRP78, a dominant regulator of the ER stress response, is increased in a variety of cancer types [25–26]. GRP78 was originally identified as a protein whose expression levels are regulated by the amount of available glucose, where hypoglycemia or aglycemia represent a strong stimulus for increased GRP78 expression [27]. Hypoglycemia, often combined with hypoxia and acidosis, represents microenvironmental conditions that are frequently present in tumor tissues and are closely aligned with increased levels of GRP78. Elevated GRP78 is among the critical pro-survival mechanisms of tumor cells to withstand and thrive under such detrimental microenvironmental conditions [28]. Over expression of GRP78 is commonly detected in malignant breast cancer tissue and is correlated with poor prognosis for these patients. Expression of GRP78 and XBP-1 was observed in 76% and 90% of breast cancers [29]. In fact, over expression of GRP78 in breast tumors predicts resistance to doxorubic in treatment in these patients [30]. Interestingly, increased expression of GRP78, ameans for tolerating low-level chronic ER stress and thriving under sub-optimal microenvironmental conditions, provides for generally increased pro-survival robustness that extends to chemotherapy, a stress condition that could not have been anticipated by evolution. It would be highly desirable to block GRP78 expression in a tumor-specific manner to reduce the ability of tumor cells to survive and proliferate in the absence of optimal nutrient supply and to increase the efficacy of cancer therapy [31].

Angiogenesis is crucial to many physiological and pathological processes, including development and cancer cell survival. PERK can also facilitate tumor growth by upregulating vascular endothelial growth factor (VEGF) and thereby inducing angiogenesis in tumors [32]. Ghosh showed that IRE1a, PERK, and ATF6a powerfully regulate VEGFA mRNA expression under various stress conditions [33]. In Ire1a2/2 and Perk2/2 mouse embryonic fibroblasts and ATF6a-knockdown HepG2 cells, induction of VEGFA mRNA by ER stress is attenuated compared to that in control cells. Embryonic lethality of Ire1a2/2 mice occurred because of a lack of VEGFA induction in labyrinthine trophoblast cells of the developing placenta. Rescue of IRE1a and PERK in Ire1a2/2 and Perk2/2 cells, respectively, prevents VEGFA mRNA attenuation. Another study showed that induction of VEGFA by IRE1a, PERK, and ATF6 involves activation of the transcription factors, spliced-XBP-1, ATF4, and cleaved-ATF6, respectively [33].

Lei revealed possible mechanisms underlying the association of ANKRD1 with cisplatin response [34]. Cisplatin-induced apoptosis in ovarian cancer cell lines was found to be associated with ER stress, as evidenced by the induction of glucose-regulated protein 78 (GRP78), growth arrest- and DNA damage-inducible gene 153 (GADD153), and increased intracellular Ca^{2+} release. The level of sensitivity to cisplatin-induced apoptosis was associated with ANKRD1 protein levels and poly (ADP-ribose) polymerase cleavage. COLO 316 ovarian cancer cells, which express high ANKRD1 levels, were relatively resistant to cisplatin, and ER stress-induced apoptosis, whereas OAW42 and PEO14 cells, which express lower ANKRD1 levels, are more sensitive to ER stress-induced apoptosis [34].

Zhou investigated anti-cancer activity in human breast cancer cell lines and explored the underlying mechanism of this action [35]. The results showed that treatment with AMP dose-dependently inhibited cell viability and induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cells without cytotoxicity in human normal breast epithelial cells MCF-10A. Additionally, AMP dose-dependently triggered ROS generation in both breast cancer cells. The ROS scavenger N-acetyl-L-cysteine strongly attenuated AMP-induced ROS production, along with cell growth inhibition and apoptosis. Furthermore, AMP was observed to activate ER stress, as evidenced by the up-regulation of ER stress-related proteins, including GRP78, p-PERK, p-elF2, cleaved-ATF6, and CHOP,

while knockdown of ATF6 or PERK markedly down-regulated AMP-induced CHOP expression. Blocking of ER stress using 4-phenylbutyric acid not only down-regulated AMP-induced GRP78 and CHOP expression, but also significantly decreased AMP-induced cell growth inhibition and apoptosis, whereas the ER stress inducer thapsigargin had opposite effects. Additionally, N-acetyl-L-cysteine inhibited AMP-induced ER stress by down-regulating GRP78 and CHOP expression. In contrast, blocking of ER stress using CHOP siRNA decreased AMP-induced ROS production and cell apoptosis [35].

Conclusion

Tumor cells are often exposed to intrinsic and external factors that alter protein homeostasis, resulting in ER stress. To cope with this, cells evoke an adaptive mechanism to restore ER proteostasis known as the UPR. Previous studies demonstrated that ER stress and UPR signaling are involved in gynecologic tumor diseases (ovarian and breast cancer). However, in tumor diseases, the roles of ER stress and UPR in cancer remains unclear, and different components are known to be involved and may be promising targets for future anticancer therapy through the activation of thepro-apoptotic GRP-78/IRE-1/XBP-1/CHOP signaling pathway.

Acknowledgment

We thank Dr. Yajun Chen of the Department of Clinical Laboratory, Nanjing Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, China, who helped us in revising the paper in English.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature, 2016, 529: 326–335.
2. Wang WA, Groenendyk J, Michalak M. Endoplasmic reticulum stress associated responses in cancer. Biochim Biophys Acta, 2014, 1843: 2143–2149.
3. Gorman AM, Healy SJ, Jäger R, *et al*. Stress management at the ER: regulators of ER stress-induced apoptosis. Pharmacol Ther, 2012, 134: 306–316.
4. Yoshida H, Matsui T, Yamamoto A, *et al*. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell, 2001, 107: 881–891.
5. Maurel M, Chevet E, Tavernier J, *et al*. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci, 2014, 39: 245–254.
6. Tirasophon W, Lee K, Callaghan B, *et al*. The endoribonuclease activity of mammalian IRE1 autoregulates its mRNA and is required for the unfolded protein response. Genes Dev, 2000, 14: 2725–2736.
7. Zhou J, Liu CY, Back SH, *et al*. The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. Proc Natl Acad Sci USA, 2006, 103: 14343–14348.
8. Glembotski CC. Endoplasmic reticulm stress in the heart. Circ Res, 2007, 101: 975–984.
9. Meeker JD, Rossano MG, Protas B, *et al*. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect, 2008, 116: 1473–1479.
10. Jheng JR, Ho JY, Horng JT, *et al*. ER stress, autophagy, and RNA viruses. Front Microbiol, 2014, 5: 388.
11. Fung TS, Liu DX. Coronavirus infection, ER stress, apoptosis and innate immunity. Front Microbiol, 2014, 5: 296.
12. Sheveleva EV, Landowski TH, Samulitis BK, *et al*. Imexon induces an oxidative endoplasmic reticulum stress response in pancreatic cancer cells. Mol Cancer Res, 2012, 10: 392–400.
13. Marciniak SJ, Garcia-Bonilla L, Hu J, *et al*. Activation-dependent substrate recruitment by the eukaryotic translation initiation factor 2 kinase PERK. J Cell Biol, 2006, 172: 201–209.
14. Harding HP, Zhang Y, Bertolotti A, *et al*. Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell, 2000, 5, 897–904.
15. Marciniak SJ, Ron D. Endoplasmic reticulum stress signaling in disease. Physiol Rev, 2006, 86: 1133–1149.
16. Kiviluoto S, Vervliet T, Ivanova H, *et al*. Regulation of inositol 1,4,5-trisphosphate receptors during endoplasmic reticulum stress. Biochim Biophys Acta, 2013, 1833: 1612–1624.
17. Nakagawa T, Zhu H, Morishima N, *et al*. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature, 2000, 403: 98–103.
18. Hitomi J, Katayama T, Eguchi Y, *et al*. Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death. J Cell Biol, 2004, 165: 347–356.
19. Chen YH, Wu XD, Yao ST, *et al*. Calcineurin is involved in cardioprotection induced by ischemic postconditioning through attenuating endoplasmic reticulum stress. Chin Med J (Engl), 2011, 124: 3334–3340.
20. Casson CN, Yu J, Reyes VM, *et al*. Human caspase-4 mediates noncanonical inflammasome activation against gram-negative bacterial pathogens. Proc Natl Acad Sci USA, 2015, 112: 6688–6693.
21. Shi J, Zhao Y, Wang Y, *et al*. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature, 2014, 514: 187–192.
22. Yamamuro A, Kishino T, Ohshima Y, *et al*. Caspase-4 directly activates caspase-9 in endoplasmic reticulum stress induced apoptosis in SH-SY5Y cells. J Pharmacol Sci, 2011, 115: 239–243.
23. Reddy RK, Mao C, Baumeister P, *et al*. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem, 2003, 278: 20915–20924.
24. Li C, Wei J, Li Y, *et al*. Transmembrane Protein 214 (TMEM214) mediates endoplasmic reticulum stress-induced caspse 4 enzyme activation and apoptosis. J Biol Chem, 2013, 288: 17908–17917.
25. Zhang Y, Bai C, Lu D, *et al*. Endoplasmic reticulum stress and autophagy participate in apoptosis induced by bortezomib in cervical cancer cells. Biotechnol Lett, 2016, 38: 357–365.
26. Banerjee A, Ahmed H, Yang P, *et al*. Endoplasmic reticulum stress and IRE-1 signaling cause apoptosis in colon cancer cells in response to andrographolide treatment. Oncotarget, 2016, 7: 41432–41444.
27. Schönthal AH. Pharmacological targeting of endoplasmic reticulum stress signaling in cancer. Biochem Pharmacol, 2013, 85: 653–666.

28. Wang WA, Groenendyk J, Michalak M. Endoplasmic reticulum stress associated responses in cancer. Biochim Biophys Acta, 2014, 1843: 2143–2149.
29. Tiwari RV, Parajuli P, Sylvester PW. ɣ-Tocotrienol-induced endoplasmic reticulum stress and autophagy act concurrently to promote breast cancer cell death. Biochem Cell Biol, 2015, 93: 306–320.
30. Lei Y, Henderson BR, Emmanuel C, *et al*. Inhibition of ANKRD1 sensitizes human ovarian cancer cells to endoplasmic reticulum stress-induced apoptosis. Oncogene, 2015, 34: 485–495.
31. Matsumura K, Sakai C, Kawakami S, *et al*. Inhibition of cancer cell growth by GRP78 siRNA lipoplex via activation of unfolded protein response. Biol Pharm Bull, 2014, 37: 648–653.
32. Blais JD, Addison CL, Edge R, *et al*. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol, 2006, 26: 9517–9532.
33. Ghosh R, Lipson KL, Sargent KE, *et al*. Transcriptional regulation of VEGF-A by the unfolded protein response pathway. PLoS One, 2010, 5: e9575.
34. Lei Y, Henderson BR, Emmanuel C, *et al*. Inhibition of ANKRD1 sensitizes human ovarian cancer cells to endoplasmic reticulum stress-induced apoptosis. Oncogene, 2015, 34: 485–495.
35. Zhou Y, Shu F, Liang X, *et al*. Ampelopsin induces cell growth inhibition and apoptosis in breast cancer cells through ROS generation and endoplasmic reticulum stress pathway. Plos One, 2014, 9: e89021.

DOI 10.1007/s10330-016-0201-1
Cite this article as: Liu KS, Fang WM, Sun EH, *et al*. Roles of endoplasmic reticulum stress and apoptosis signaling pathways in gynecologic tumor cells: A systematic review. Oncol Transl Med, 2017, 3: 131–135.

Oncology and Translational Medicine

Aims & Scope

Oncology and Translational Medicine is an international professional academic periodical. The Journal is designed to report progress in research and the latest findings in domestic and international oncology and translational medicine, to facilitate international academic exchanges, and to promote research in oncology and translational medicine as well as levels of service in clinical practice. The entire journal is published in English for a domestic and international readership.

Copyright

Supervised by

Ministry of Education of the People's Republic of China.

Administered by

Tongji Medical College, Huazhong University of Science and Technology.

Submission information

Manuscripts should be submitted to:
http://otm.tjh.com.cn
dmedizin@sina.com

Subscription information

ISSN edition: 2095-9621
CN: 42-1865/R

■ Subscription rates
Subscription may begin at any time. Remittances made by check, draft or express money order should be made payable to this journal. The price for 2017 is as follows: US $ 30 per issue; RMB ￥28.00 per issue.

Database

Oncology and Translational Medicine is abstracted and indexed in EMBASE, Index Copernicus, Chinese Science and Technology Paper Citation Database (CSTPCD), Chinese Core Journals Database, Chinese Journal Full-text Database (CJFD), Wanfang Data; Weipu Data; Chinese Academic Journal Comprehensive Evaluation Database.

Business correspondence

All matters relating to orders, subscriptions, back issues, offprints, advertisement booking and general enquiries should be addressed to the editorial office.

Mailing address
Editorial office of
Oncology and Translational Medicine
Tongji Hospital
Tongji Medical College
Huazhong University of Science and Technology
Jie Fang Da Dao 1095
430030 Wuhan, China
Tel.: +86-27-83662630
Fax: +86-27-83662645
Email: dmedizin@tjh.tjmu.edu.cn

Printer
Changjiang Spatial Information Technology Engineering Co., Ltd. (Wuhan)
Hangce Information Cartorgraphy Printing Filial, Wuhan, China
Printed in People's Republic of China

Managing director

Executive editors