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Original Article

Clinical study on personalized treatment for advanced NSCLC guided by ERCC1 protein detection

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Abstract: Background & Objective: Excision repair cross complementing 1 (ERCC1) participates in the resistance of non-small cell lung cancer (NSCLC) to platinum-based chemotherapy drug. This study aimed to explore the role of ERCC1 protein expression in personalized treatment for advanced NSCLC patient and its significance. Method: From January 2010 to December 2011, 159 advanced stage (stage IIIB-IV) NSCLC patients were enrolled. The expression of ERCC1 protein in lung cancer tissue of the patient was detected by immunohistochemical method. In a ratio of 2:1, patients were randomly divided into either the personalized treatment group or the standard treatment group. The standard treatment group adopted the platinum-based chemotherapy regimen, namely, gemcitabine/cisplatin or navelbine/cisplatin. In personalized treatment group, patients with high ERCC1 protein expression received gemcitabine/navelbine, and those with low ERCC1 protein expression received gemcitabine/cisplatin or navelbine/cisplatin. The main observed indices included response rate, overall survival and time to progression. Group comparison was conducted by chi-square test. Comparison of one-year survival rate and survival was conducted by Life table and Kaplan-Meier method. Results: Follow-up data were up to December 31, 2014. The response rate of the standard treatment group and the personalized treatment group was 26.4% and 27.4%, respectively. The difference of the two groups was not statistically significant (P=0.899). The median survival was 9.4 months (95% CI was 7.88-10.92 months) in the standard treatment group and 13.2 months (95% CI was 12.34-14.06 months) in the personalized treatment group. The difference of two groups was statistically significant (P=0.045). The time to progression was 5.0 months (95% CI was 3.84-6.16 months) in the standard treatment group and 4.7 months (95% CI was 4.03-5.37 months) in the personalized treatment group, without significant difference (P=0.369). The one-year survival rate of the standard treatment group and the personalized treatment group was 41.5% and 46.2%, respectively, without significant difference (chi-square value=0.318, P=0.572). Conclusion: Compared with the standard treatment group, the median survival of the personalized treatment group is extended. However, the personalized treatment for advanced NSCLC guided by ERCC1 protein detection does not show advantages in response rate, survival and time to progression. Additional clinical studies are needed to optimize the detection of biomarkers so as to guide reasonable selection of clinical chemotherapy regimens.

Keywords: Cancer, non-small cell lung, protein expression, personalized treatment, excision repair cross complementing 1 (ERCC1)

Introduction

Lung cancer is a kind of tumor with the highest morbidity and mortality in the world. About 80%-85% lung cancer belongs to non-small cell lung cancer (NSCLC). Due to the lack of effective means of diagnosis in early stage, about 75% patients miss the opportunity of surgery at the first visit. Presently, the main therapy for advanced NSCLC is combined chemotherapy, but the 5-year survival rate is less than 15% [1]. Many studies show that the main reason influencing chemotherapy outcome and survival of NSCLC patients is the tumor cells' resistance to anticancer drug. A recent study [2] indicated that abnormal DNA repair of tumor cells and abnormal expression of related gene were closely related to the generation of drug resistance of lung cancer. Prospective detection of molecular markers contributes to making personalized treatment plan and may become the key to improve chemotherapy response.

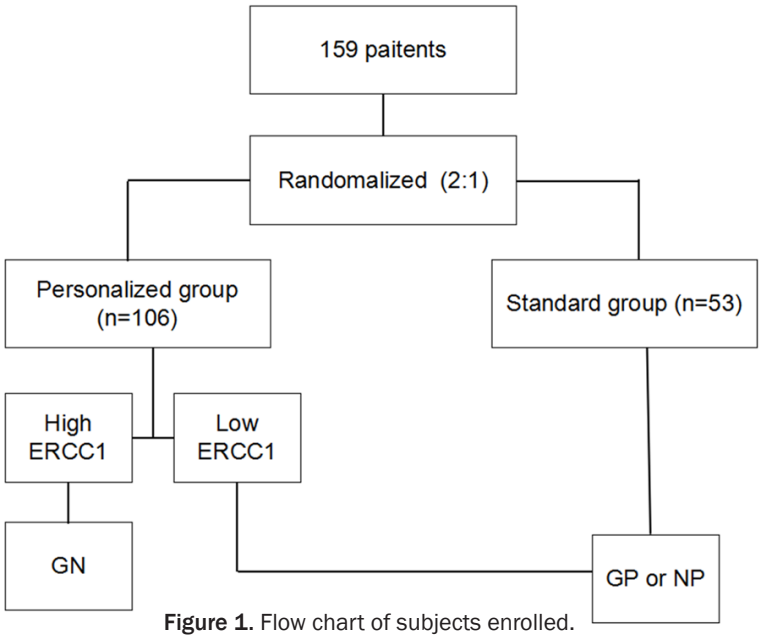

Figure 1. Flow chart of subjects enrolled.

Excision repair cross-complementing 1 (ERCC1) is associated with NSCLC's resistance to platinum chemotherapy drug. Expression of ERCC1 is closely related to chemotherapy response and prognosis of NSCLC [3]. This study explored the role of ERCC1 protein detection in personalized treatment for advanced NSCLC patients and its significance.

Materials and methods

Subjects

As shown in **Figure 1**, advanced stage (stage IIIB-IV) NSCLC patients, which were admitted in Shanghai Chest Hospital Shanghai Jiaotong University from January 2010 to December 2011, did not receive chemotherapy previously and cannot receive excision, were recruited as the subjects. All patients were confirmed by histopathological diagnosis through bronchoscopic biopsy or needle biopsy under CT positioning. The pathological specimens and clinical data were collected. A total of 159 cases (102 males, 57 females) were included, which were aged 36-75 years, with the median age of 60 years. There were 40 cases with squamous carcinoma, 107 cases with adenocarcinoma, 3 cases with adenosquamous carcinoma, 1 case with large cell carcinoma and 8 cases with undefined cancer. All patients had measurable tumor foci. According to 2009 TNM Staging of UICC, there were 61 cases in stage IIIb and 98 cases in stage IV. The last follow-up was December 31, 2014.

Immunohistochemistry

The NSCLC biopsy specimens of 106 cases, obtained through bronchofiberscopy or lung puncture, were collected. Corresponding paraffin block was selected. The thickness of serial paraffin section was 4 μm. IHC detection was conducted as per EnVision method. Conventional deparaffinization and hydration were conducted. Heating pH 6.0 citric acid was conducted for antigen retrieval. 3% H_2O_2 was used to block peroxidase. 50 μL/section primary antibody (Mouse anti-human ERCC1 antibody was diluted by TBS as per 1:80 and purchased from Abcom) was added at 4°C overnight. 50 μL/section second antibody was added at room temperature for 60 min. DAB color development was performed for 5-10 min. The section was washed by TBS buffer solution for 3 times (5 min/time) between each step. Hematoxylin counterstaining, drying and sealing were performed.

The immunohistochemical result was judged by two senior pathologists independently based on the standard of Wachters et al. [4]. The section was observed under a 400X light microscope. ERCC1 positive staining was located at nuclei. Under the microscope, the cell which had clear cell structure, a nucleolus with granular brown deposition and obvious staining compared to the background was judged as positive expression. No staining of the tumor nucleus or the number of stained positive cells <10.0% was judged as negative (-). Stained positive cells ≥10.0% were judged as positive (+). The known positive section was used as positive control. Primary antibody was replaced by TBS buffer solution for the negative control.

Personalized treatment regimen

In a ratio of 2:1, the patients were randomly divided into either the personalized treatment group (106 cases) or the standard treatment

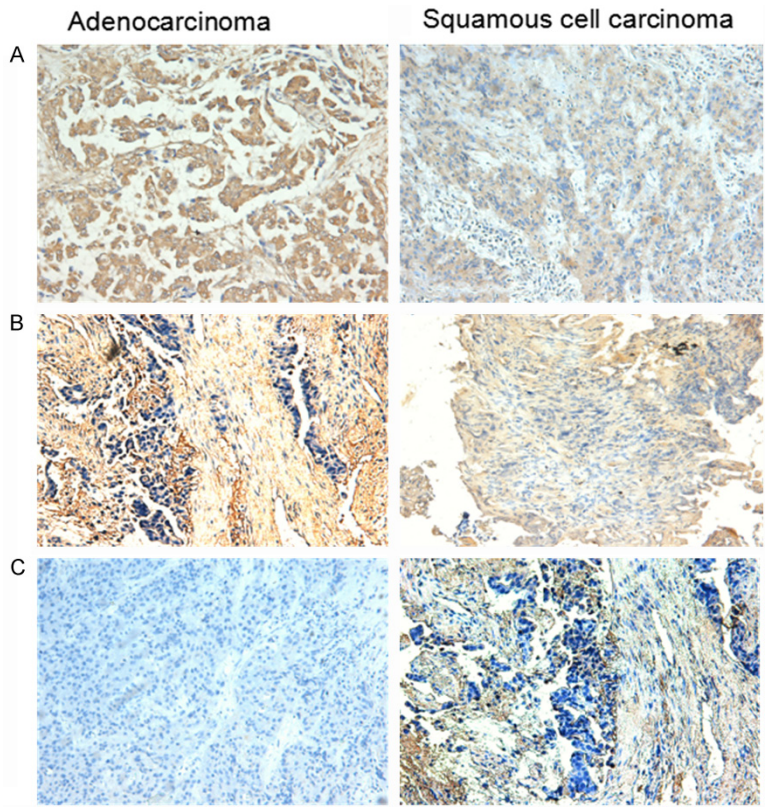

Figure 2. Expression of ERCC1 in NSCLC tissues. A: High expression; B: Low expression; C: Negative. (SP, ×200).

group (53 cases). The standard treatment group adopted the chemotherapy regimen containing platinum--GP or NP. NP: Navelbine 25 mg/m^2 D1, D8+Cisplatin 75 mg/m^2 D1, 28 days a cycle. GP: Gemcitabine 1 250 mg/m^2 D1, D8+Cisplatin 75 mg/m^2 D1, 28 days a cycle. In the personalized treatment group, patients with high ERCC1 protein expression received non-platinum chemotherapy regimen GN: Navelbine 25 mg/m^2 D1, D8+Gemcitabine 1 250 mg/m^2 D1, D8, 28 days a cycle. Patients with high ERCC1 protein expression received platinum-based chemotherapy regimen GP or NP. NP: Navelbine 25 mg/m^2 D1, D8+Cisplatin 75 mg/m^2 D1, 28 days a cycle, GP: Gemcitabine 1 250 mg/m^2 D1, D8+Cisplatin 75 mg/m^2 D1, 28 days a cycle.

Efficacy evaluation

The main observed indices included response rate (RR), overall survival (OS) and time to progression (TTP). Complete remission (CR), partial remission (PR), minor remission (MR), stable disease (SD) and progressive disease (PD) were defined according to the standard of WHO and UICC. CR+PR+MR meant objective response in short term. CR+PR+MR+SD meant beneficial response. The response case was checked and confirmed one month later. TTP referred to the interval from the beginning of treatment to progression of the tumor focus. OS referred to the interval from the beginning of treatment to death or the last follow-up. The performance status was scored by ECOG-PS of Eastern Cooperative Oncology Group (ECOG).

Statistical analysis

SPSS 22.0 software was used to perform data analysis. Measurement data were presented as $\bar{x}\pm S$. Chi square test was used for intergroup comparison, with the significant level $\alpha=0.05$. Kaplan-Meier method was adopted for survival analysis. Log-rank test was employed for survival rate comparison, with significant level $\alpha=0.05$. $P<0.05$ indicated significant difference.

Results

Expression of ERCC1 protein in advanced NSCLC tissue and clinical features of patients in each group

Immunohistochemical method was used to detect the ERCC1 protein expression in pathological specimens of 106 advanced NSCLC patients (the personalized treatment group) receiving initial treatment. High ERCC1 protein expression was found in 50 cases, while low ERCC1 protein expression was found in 56 cases (see **Figure 2**). The detailed clinical features of 53 patients in the standard treatment group and 106 patients in the personalized treatment group are listed in **Table 1**, including sex, age, performance status score, TNM stage, pathological types and differentiation degree, etc.

Table 1. Baseline patient characteristics according to treatment arm (N=159)

Characteristic	Standard treatment group (n=53)		Personalized treatment group (n=106)					
			Total		ERCC1 low expression Subgroup		ERCC1 high expression Subgroup	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	24	45.3	78	73.6	45	80.4	33	66.0
Female	29	54.7	28	26.4	11	19.6	17	34.0
Age range, years	62 (38-74)		59 (36-75)		58 (38-75)		61 (36-73)	
Mean ± SD	60.2±10.3		58.2±10.1		58.9±10.3		57.4±11.1	
ECOG PS								
0	22	41.5	48	45.3	28	50.0	20	40.0
1	30	56.6	45	42.5	19	33.9	26	52.0
2	1	1.9	13	12.2	9	16.1	4	8.0
Disease Stage								
IIIB	15	28.3	46	43.4	25	44.6	21	42.0
IV	38	71.7	60	56.6	31	55.4	29	58.0
Histological type								
Adenocarcinoma (Ad)	41	77.3	66	62.3	34	60.7	32	64.0
Squamous cell carcinoma (Sq)	8	15.1	32	30.2	17	30.3	15	30.0
Ad-Sq	1	1.9	2	1.9	1	1.8	1	2.0
Large cell carcinoma	0	0.0	1	0.9	1	1.8	0	0.0
NOS	3	5.7	5	4.7	3	5.4	2	4.0
Differentiation degree of Ad								
Poorly deferentiated	12	29.3	14	21.2	7	20.6	7	21.9
Moderately/well differentiated	29	70.7	52	78.8	27	79.4	25	78.1
Differentiation degree of Sq								
Poorly deferentiated	1	12.5	11	34.4	5	29.4	6	40.0
Moderately/well differentiated	7	87.5	21	65.6	12	70.6	9	60.0

Note: Personalized group vs. standard group : sex, P=0.054; age, P=0.324; ECOG PS, P=0.192; Disease Stage, P=0.065; Histological type, P=0.056; Differentiation degree of Ad, P=0.894; High ERCC1 expression vs. low ERCC1 group: sex, P=0.094; age, P=0.467; ECOG PS, P=0.206; Disease Stage, P=0.784; Histological type, P=0.727; Differentiation degree of Ad, P=0.528.

Chemotherapy response, one-year survival rate, median survival and time to progression of each treatment group

As shown in **Table 2**, in terms of chemotherapy response, the response rate of the standard treatment group and the personalized treatment group was 26.4% and 27.4%, respectively, without significant difference (*P*=0.899). In the personalized group, the response rate of the high ERCC1 expression group was 26.0% and the low ERCC1 expression group was 28.6%, the difference was not significant (P=0.767). The one-year survival rate of the standard treatment group and the personalized treatment group was 41.5% and 46.2%, respectively, without significant difference (chi-square value=0.318, *P*=0.572).

The median survival was 9.4 months (95% CI was 7.88-10.92 months) in the standard treatment group and 13.2 months (95% CI was 12.34-14.06 months) in the personalized treatment group, with significant difference (**Figure 3A**, *P*=0.045).

The median survival was 11 months (95% CI was 7.68~14.32 months) in the ERCC1 high expression group and 13.9 months (95% CI was 12.40~15.40 months) in the ERCC1 low expression group, without significant difference (**Figure 3B**, P=0.303).

Table 2. Outcome according to treatment arms

Outcome	Standard treatment group (n=53)		Personalized treatment group (n=106)					
			Total		ERCC1 low expression Subgroup (n=56)		ERCC1 high expression Subgroup (n=50)	
	Case	% or 95% CI	Case	% or 95% CI	Case	% or 95% CI	Case	% or 95% CI
Response								
CR	1	1.9	0	0.0	0	0.0	0	0.0
PR	13	24.5	29	27.4	16	28.6	13	26.0
SD	21	39.6	39	36.8	25	44.6	14	28.0
PD	18	34	38	35.8	15	26.8	23	46.0
Survival								
Median, months	9.4	7.88 to 10.92	13.2*	12.34 to 14.06	13.9	12.40 to 15.40	11	7.68 to 14.32
1 year (%)	41.5		46.2		48.2		44.0	
Median TTP, months	5.00	3.84 to 6.16	4.70	4.03 to 5.37	4.90	4.25 to 5.55	3.80	2.88 to 4.72

Note: CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease. *compared to standard treatment group, P=0.045.

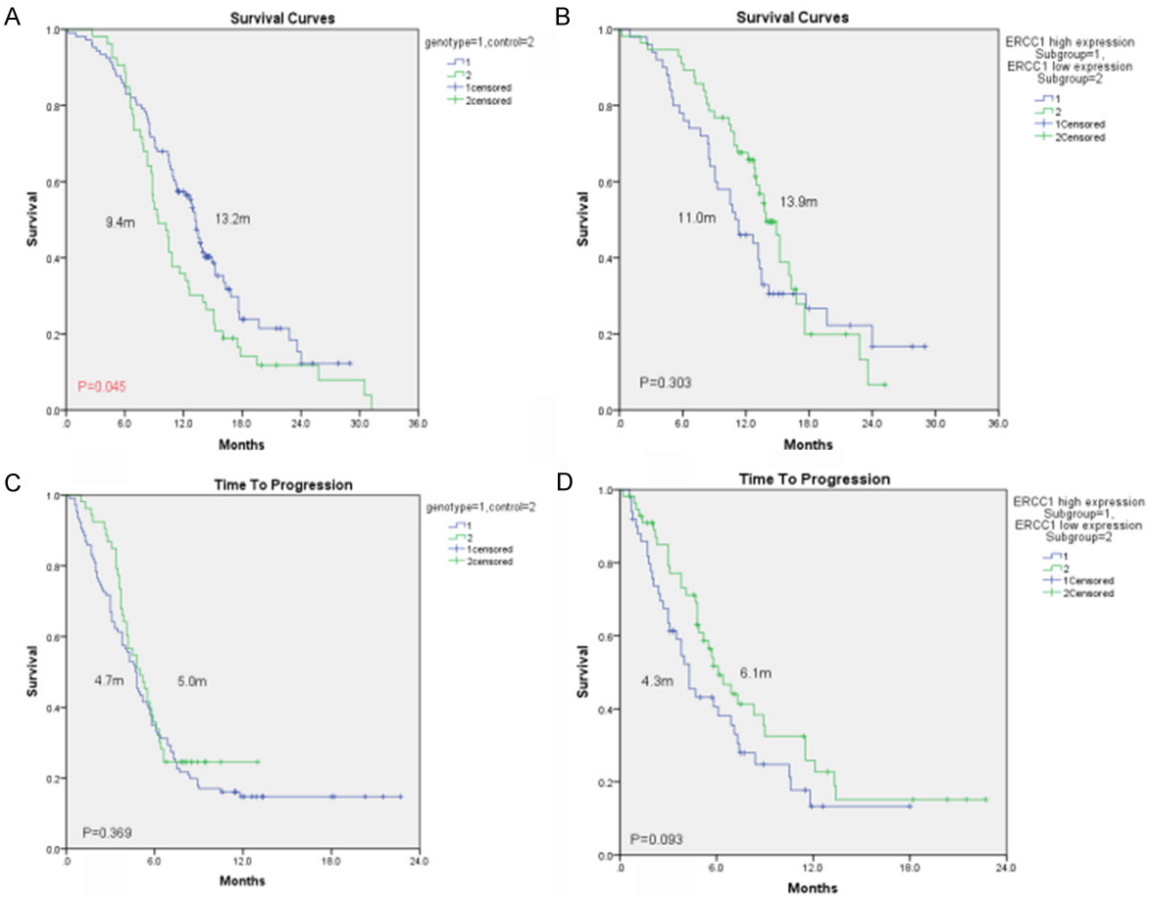

Figure 3. Time to progression and survival curves of NSCLC patients. A: Survival curves (Personalized treatment group vs. standard treatment group); B: Survival curves (High ERCC1 expression group vs. low ERCC1 expression group); C: Time to progression curves (Personalized treatment group vs. standard treatment group); D: Time to progression curves (High ERCC1 expression group vs. low ERCC1 expression group).

The time to progression was 5.0 months (95% CI was 3.84-6.16 months) in the standard treatment group and 4.7 months (95% CI was 4.03-5.37 months) in the personalized treat-

ment group. The difference of the two groups was not statistically significant (P=0.369) (**Figure 3C**).

In the personalized group, the time to progression was 4.3 months (95% CI was 3.22~5.38 months) in the high ERCC1 expression group and 6.1 months (95% CI was 4.48~7.72 months) in the low ERCC1 expression group. The difference of the two groups was not statistically significant (P=0.093) **Figure 3D**).

Discussion

Studies in recent years showed that abnormal expression of cell signal transduction related genes, abnormal DNA repair of tumor cells and abnormal expression of other related genes are closely related to drug resistance of lung cancer [5]. This indicates that prospective detection of molecular marker is the key of personalized treatment and improving chemotherapy response. Located on Chromosome 19, ERCC1 is an important member of nucleotide excision repair family and a protein encoding 297 amino acids. ERCC1 forms heterodimer with XPF, excises at 5' terminal--damaged part of DNA single strand and plays its role. ERCC1 overexpression can rapidly repair the damaged DNA of cell stagnating at G2/M phase, resulting in cellular resistance to cisplatin.

Ceppi et al. [6] detected the expression level of ERCC1 mRNA in tissues of 70 NSCLC patients. They found that patients with low ERCC1 mRNA expression had long median survival (17.3 months *vs.* 10.9 months, P=0.0032). Moreover, in patients receiving cisplatin treatment, low expression level of ERCC1 was an important prognostic factor for long survival (23.0 months *vs.* 12.4 months, P=0.0001). Simon et al. [7] conducted a prospective phase II study, and drug for advanced NSCLC was selected based on the expression level of ERCC1 and RRM1 gene. Patients with low ERCC1 expression received platinum chemotherapy, and those with high ERCC1 expression received non-platinum chemotherapy. For 60 patients completing treatment, the response rate was 44%; the median survival was 13.3 months; the one-year survival rate was 59%. These results were significantly superior to the outcome of standard platinum-based two-drug combination scheme. The results of this study showed that the response rate of the personalized treatment group (27.4%), in which chemotherapy regimen was selected based on the expression of ERCC1 protein, was slightly higher than that of the standard treatment group (26.4%). The difference of the two groups was not statistically significant (P=0.899).

Cobo et al. [8] conducted a multi-center randomized controlled phase III trial, in which platinum drug was selected based on ERCC1 mRNA expression. 444 stage IV NSCLC patients were randomly assigned to either experimental group or control group in a ratio of 2:1. The control group adopted the regimen of cisplatin/docetaxel. For experimental group, drug was selected based on the expression of ERCC1 mRNA. Cisplatin/docetaxel was used for patients with low ERCC1 expression, and the non-platinum chemotherapy regimen--docetaxel/gemcitabine was used for patients with high ERCC1 expression. The main endpoint criteria were objective response rate. In the evaluable patients, the response rate of experimental group was 50.7% which was obviously superior to 39.3% in control group (P=0.02). In experimental group, the response rate was 53.2% in patients with low ERCC1 expression and 47.2% in patients with high ERCC1 expression, both reaching the main study endpoint. In terms of progression free survival--the secondary endpoint criteria, experimental group (6.1 months) showed the trend of extension compared with control group (5.2 months). This showed that the selected platinum drug use based on the expression level of ERCC1 mRNA was superior to traditional two-platinum drug treatment pattern without selection. However, some problems in Cobo's study are worthy of discussion. For example, in the aspect of study design, there is no retrospective analysis on the relationship between ERCC1 mRNA expression in control group and efficacy of cisplatin/docetaxel regimen. Therefore, we cannot judge whether replacing cisplatin/docetaxel with gemcitabine/docetaxel for the population with high ERCC1 mRNA expression is reasonable or not.

In the phase III clinical trial conducted by Bepler et al. [9], the predictive effect of ERCC1 mRNA did not show clinical benefit. However, Bepler et al. [10] conducted microdissection for paraffin-embedded section of the above trial, so as to extract RNA and analyze the expression level of

ERCC1 mRNA with primer and probe for commercial verification. The median and critical value of optimization was verified in another large randomized phase III trial (trial B, Ann Oncol 25: 2147-55, 2014). The results indicated that the expression level of ERCC1 mRNA (detected by commercial CLIA/CAP certification) can predict the benefit of advanced NSCLC patients receiving gemcitabine/cisplatin (GP) chemotherapy regimen.

In the present study, the expression of ERCC1 protein in lung cancer tissue of the patient was detected by immunohistochemical method. The patients were randomly assigned to either personalized treatment group or standard treatment group in a ratio of 2:1. The standard treatment group adopted the platinum-based chemotherapy regimen, namely, gemcitabine/cisplatin or navelbine/cisplatin. In the personalized treatment group, patients with high ERCC1 protein expression received navelbine/gemcitabine (non-platinum chemotherapy regimen), and those with low ERCC1 protein expression received gemcitabine/cisplatin or navelbine/cisplatin (platinum-based chemotherapy regimen). The results showed that the one-year survival rate of the personalized treatment group (46.2%) was higher than that of standard treatment group (41.5%), but the difference of the two groups was not statistically significant (chi-square value=0.318, P=0.572). The median survival of the personalized treatment group was longer than that of the standard treatment group (13.2 months *vs*. 9.4 months), with significant difference (P=0.045). The reason may be that the second-line treatment of the patients was not controlled in the present study. The difference of the second-line treatment and follow-up treatment (such as targeted drug therapy) after progressive disease had a great influence on survival of the patients.

Although many studies indicate that the expression level of ERCC1 protein can predict the efficacy of platinum-based regimen, whether the advantage brought by methodology can be turned into the feasibility of clinical practice remains to be verified by prospective study. Moreover, different detection methods of ERCC1 expression in each study and different division standards of ERCC1 expression level restrict the application of markers such as ERCC1 to the selected drug use in clinical practice. Therefore, how to set cut-off point for high and low expression of molecular markers is important in guiding prospective personalized treatment with expression level of marker.

Disclosure of conflict of interest

None.

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