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ORIGINAL ARTICLE

Clinical evaluation of postoperative chemotherapy based on genetic testing in patients with stage IIIA non-small cell lung cancer

Jinbai Miao, Wenqian Zhang, Xiaoxing Hu, Shuo Chen, Bin Hu & Hui Li

Department of Thoracic Surgery, Beijing Chao-Yang Hospital Affiliated Capital Medical University, Beijing, China

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Correspondence
Hui Li, Beijing Chao-Yang Hospital, Capital Medical University, 8 GongrenTiyuchangNanlu, Chao-Yang District, Beijing 100020, China.
Tel: +86 10 85231454
Fax: +86 10 85231165
Email: huilee@vip.sina.com

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Abstract

Background: We performed a retrospective analysis to evaluate whether a postoperative chemotherapy selection method based on four tumoral gene expression tests would improve prognosis in patients with stage IIIA non-small cell lung cancer (NSCLC) after surgery.

Methods: Between January 2007 and July 2011, 148 patients with stage IIIA NSCLC underwent radical lobectomy with four cycles of adjuvant postoperative chemotherapy. Forty-five patients had tailored treatment plans based on the results of tumoral gene expression tests. The tests consisted of quantitative real-time polymerase chain reaction analyses to measure the messenger ribonucleic acid levels of the excision repair cross-complementing gene 1, ribonucleotide reductase Ml, type III β-tubulin, and thymidylate synthase genes in tumor tissues. One hundred and three patients received conventional chemotherapy. Disease responses were assessed after two cycles and every three months after the first four cycles of chemotherapy. The one and two-year survival rates and diesease-free survival (DFS) rates were recorded, and the adverse effects documented.

Results: The one and two-year DFS rates in the genetically tested group were better than those in the non-tested group, and the differences were statistically significant ($P < 0.05$). The two-year Kaplan–Meier DFS curve analysis results were significantly better in the genetically tested group ($X^2 = 8.228$, $P = 0.004$). The adverse effects during the treatments were not significantly different ($P > 0.05$) between the two groups.

Conclusions: The chemotherapy selection method based on four tumoral gene expression tests demonstrated its feasibility to improve the efficacy of adjuvant postoperative chemotherapy and benefit stage IIIA NSCLC patients by yielding better DFS without increasing the adverse effects of chemotherapy.

Introduction

Lung cancer has the highest morbidity and mortality rates worldwide, with non-small cell lung cancer (NSCLC) representing 80% of cases.[1,2] The most common treatment plan for stage IB-IIIA NSCLC is surgical resection combined with postoperative adjuvant chemotherapy, according to the disease stage.[3] Currently, the selection of doublet chemotherapy for the majority of patients is typically determined based on the oncologist's personal preference, the convenience of delivery, and regimen-specific toxicity. The standard adjuvant chemotherapy regimens for NSCLC combine platinum-based drugs with other third generation anti-tumor drugs (vincristine, paclitaxel, gemcitabine, or pemetrexed), but the overall response rate is only 25–35%, which could be a result of intrinsic or induced tumoral drug resistance during treatment.[4] Recently, several reports have suggested that tailored individual chemotherapy based on tumoral genetic testing is predictive in advanced NSCLC.[5–7]

The expression levels of several genes in tumor samples have been found to be associated with certain levels of drug resistance. For example, excision repair cross-complementing gene 1 (ERCC1) is a DNA damage repair gene that encodes the 5' endonuclease of the nuclear excision repair complex (NER) and plays an important role in DNA damage repair. Platinum compounds can form adducts with

and cross-links between DNA molecules and, thus, effectively block DNA replication. Repair of these adducts and cross-links are dependent on the NER complex; thus, high levels of ERCC1 gene expression trigger resistance to platinum agents.[8,9] The ribonucleotide reductase M1 (RRM1) gene is the regulatory subunit of ribonucleotide reductase, and the expression level of the RRM1 gene is also the predominant cellular determinant of chemotherapeutic efficacy for gemcitabine.[9,10] Similarly, a high expression level of the thymidylate synthase (TYMS) gene is associated with pemetrexed resistance.[11,12] Recently, the expression level of the type III β-tubulin (TUBB3) gene was shown to be associated with vincristine and paclitaxel chemotherapy resistance.[13,14]

In the past decade, tailored therapy has made unprecedented progress in the treatment for NSCLC. However, most reports focus upon non-operative advanced NSCLC, some with different and even contradictory results. This retrospective study was performed to evaluate the efficacy of customized chemotherapy regimens based on the tumoral messenger ribonucleic acid (mRNA) levels of four genes (ERCC1, RRM1, TYMS, and TUBB3) in stage IIIA NSCLC postoperative patients to improve clinical outcomes.

Methods

Patient enrollment

For this study, we selected 148 from 235 patients who were diagnosed with stage IIIA NSCLC and had received radical lobectomy in the Thoracic Surgery Department of Beijing Chao-Yang Hospital from January 2007 to July 2011. Of the 87 ineligible patients, 26 received tyrosine kinase inhibitors (TKIs) as first line treatment after surgery based on positive epidermal growth factor receptor (EGFR) testing. TKIs as second line treatment after disease progression meant that 30 patients were ineligible for the study. The other 31 patients were ineligible as a result of incomplete data.

Among 148 patients, there were 97 men and 51 women. The ages of the patients ranged from 28–79 years (median, 60.8 years). The patients were divided into tested (n = 45) and non-tested groups (n = 103) according to the patients' agreement to undergo chemotherapy sensitivity genetic testing. All patients underwent complete anatomical lobectomy, with systematic mediastinal lymph node dissection in open surgery and no complications occurred. The institutional review board of Beijing Chao-Yang Hospital approved the study and the electronic database used. Patient consent was obtained for entry into the database, and patients were aware that this information would be used for research purposes.

Chemotherapy regimen

The tumoral mRNA levels of the ERCC1, RRM1, TUBB3, and TYMS genes in the tested group of patients were measured by fluorescent real-time polymerase chain reaction (PCR) using the Biomark Kit (ACCB Biotech Ltd, Beijing, China). Predetermined values for these genes, which were generated from large cohorts of Chinese patients, were used to dichotomize expression levels following the manufacturer's instructions.

Table 1 The first round of selection was based on the expression levels of ERCC1 and RRM1

First round	RRM1 (low)	RRM1 (high)
ERCC1 (low)	Carboplatin + gemcitabine (11/45)	Carboplatin + paclitaxel (9/45)
ERCC1 (high)	Gemcitabine + paclitaxel (7/45)	To be determined in second round

ERCC1, excision repair cross-complementing gene 1; RRM1, ribonucleotide reductase M1.

The chemotherapy regimen was determined according to the following strategy in the tested group of patients. The first round of selection was based on the expression levels of ERCC1 and RRM1 (Table 1). In the ERCC1 (high)/RRM1 (high) subgroup of patients, the second round of selection was based on the expression levels of TYMS and TUBB3 (Table 2).

The patients in the non-tested group were randomly assigned to a treatment plan with carboplatin combined with gemcitabine or paclitaxel.

The above selection strategy resulted in the following treatment plans in this study:

Carboplatin + paclitaxel: 150 mg/m^2 paclitaxel on day one, and carboplatin, area under the curve (AUC) five on day one, every 21 days;

Carboplatin + gemcitabine: 1000 mg/m^2 gemcitabine on days one and eight, and carboplatin, AUC five on day one, every 21 days;

Gemcitabine + paclitaxel: 1000 mg/m^2 gemcitabine on days one and eight, and 120 mg/m^2 paclitaxel on day one, every 21 days;

Pemetrexed + paclitaxel: 500 mg/m^2 pemetrexed on day one, and 120 mg/m^2 paclitaxel on day one, every 21 days;

Pemetrexed only: 500 mg/m^2 pemetrexed on day one, every 21 days;

Table 2 The second round of selection was based on the expression levels of TYMS and TUBB3

ERCC1 (high) RRM1 (high)	TUBB3 (low)	TUBB3 (high)
TYMS (low)	Pemetrexed+ paclitaxel (7/45)	Pemetrexed only (3/45)†
TYMS (high)	Docetaxel only (5/45)	Carboplatin + gemcitabine or paclitaxel (3/45)

†3cases are adenocarcinoma. ERCC1, excision repair cross-complementing gene 1; TUBB3, type III β-tubulin; TYMS, thymidylate synthase.

Docetaxel only: 80 mg/m^2 docetaxel on day one, every 21 days.

Disease responses in all of the patients were monitored after two cycles and every three months after four cycles of chemotherapy. The one and two-year survival and disease-free survival (DFS) rates were recorded and adverse effects documented.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (version 17, SPSS Inc., Chicago, IL, USA). Overall survival (OS) and DFS were estimated using the Kaplan–Meier method, and differences in OS and PFS between the study groups were assessed by a two-sided log-rank test. $P < 0.05$ was considered statistically significant.

Results

Patients

The patients were divided into tested (n = 45) and non-tested groups (n = 103) according to the patients' agreement to undergo chemotherapy sensitivity genetic testing. Characteristics of the 148 patients are listed in Table 3. All patients received open lobectomy and systematic lymph node dissection. There was no significant difference in surgical sites and the number of dissected lymph nodes between the two groups (Table 3). Among the 148 NSCLC cases, 80 were squamous cell carcinoma and the remaining 68 cases were adenocarcinoma. The pathological tumor node metastasis (TNM) stages of these patients were: 78 cases of T3N1M0 (52.7%), 26 cases of T1-2N2M0 (17.6%), and 44 cases of T3N2M0 (29.7%). No significant differences between the two groups were found (Table 3).

Table 3 Patient demographics and disease characteristics

Variable	Tested group	Non-tested group	*P**
Number	45	103	
Age at diagnosis, years			0.991
Range	29–75	28–79	
Mean	60.8	60.9	
Gender			0.346
Male	32	65	
Female	13	38	
Operation			0.727
Upper lobectomy	13	33	
Low lobectomy	22	55	
Middle lobectomy	8	12	
Low-middle lobectomy	2	3	
Dissected lymph node number	24.4 ± 5.80	23.1 ± 5.50	0.189
ECOG performance status after operation (0/1/2)	43/0/0	103/0/0	
Pathology			0.405
Squamous cell carcinoma	22	58	
Adenocarcinoma	23	45	
Stage			0.552
T3N1M0	21	57	
T1-2N2M0	8	18	
T3N2M0	16	28	
Metastasis sites			0.832
Bone	5	18	
Liver	3	8	
Lung	13	36	
Brain	2	11	
Subcutaneous	0	2†	

**P* value for age was from Independent-Samples *t*-test; all others from chi-square test. † One case under the scalp, one case under the left lower limb skin. ECOG, Eastern Cooperative Oncology Group.

Overall survival and disease-free survival

The one-year OS rate was slightly higher in the tested group (84.4%) compared with the non-tested group (80.6%), and the difference in the two-year OS rate was even greater between the tested (68.9%) and the non-tested group (61.2%). However, the difference in either OS rate was not significant between the groups (Table 4). The median follow-up periods were 700 days (95% confidence interval [CI]: 637, 763) for the tested group and 300 days (95% CI: 207 393) for the non-tested group.

Encouragingly, the tested group had a significantly better DFS rate than the non-tested group (Table 4). The tested group (66.7%) had a higher one-year DFS rate than the non-tested group (44.7%), and the difference was significant (X^2 = 6.071, P = 0.014). The two-year DFS rates in the tested and non-tested groups were 48.9% and 27.2%, respectively (X^2 = 6.595, P = 0.010). We also performed Kaplan–Meier survival curve analysis (Fig 1) and found that the DFS rate in the tested group was significantly higher than in the non-tested group (X^2 = 8.228, P = 0.004). These results suggested that this four-gene expression-based customized chemotherapy regimen could improve DFS in stage IIIA NSCLC patients.

Further stratified analyses according to TNM stage also showed that the DFS rate in the tested group was significantly higher than in the non-tested group in T3N1M0 patients (Table 5). The one-year DFS rates in the two groups were 76.2% and 49.1% (X^2 = 4.573, P = 0.032), respectively, and the two-year DFS rates were 57.1% and 33.3%, respectively (X^2 = 5.178, P = 0.023). The OS rate was slightly higher in the tested group, but the difference was not significant (Table 5). Neither the OS nor the DFS rates were significantly different between the groups among all of the T1-3N2M0 patients (Tables 5, 6).

Adverse effects

The main adverse effects during the treatments included bone marrow suppression, gastrointestinal reactions, and

Table 4 Comparison of the one and two-year overall and disease-free survival rates between the tested and non-tested groups

	Tested group	Non-tested group	X^2	*P*
One-year overall survival	0.844 (38/45)	0.806 (83/103)	0.313	0.567
Two-year overall survival	0.689 (31/45)	0.612 (63/103)	0.806	0.369
One-year disease-free survival	0.667 (30/45)	0.447 (46/103)	6.071	0.014
Two-year disease-free survival	0.489 (22/45)	0.272 (28/103)	6.595	0.010

liver and renal dysfunctions. The incidence of nausea/vomiting was 75.6% in the tested group and 84.5% in the non-tested group, but the difference was not significant ($X^2 = 1.667$, $P = 0.197$). A decline in white blood cell count occurred in 46.7% of patients in the tested group and in 52.4% of patients in the non-tested group, but the difference was also not significant ($X^2 = 0.416$, $P = 0.519$). We concluded that our customized chemotherapy regimen would not affect the overall rate of adverse effects in stage IIIA NSCLC postoperative patients.

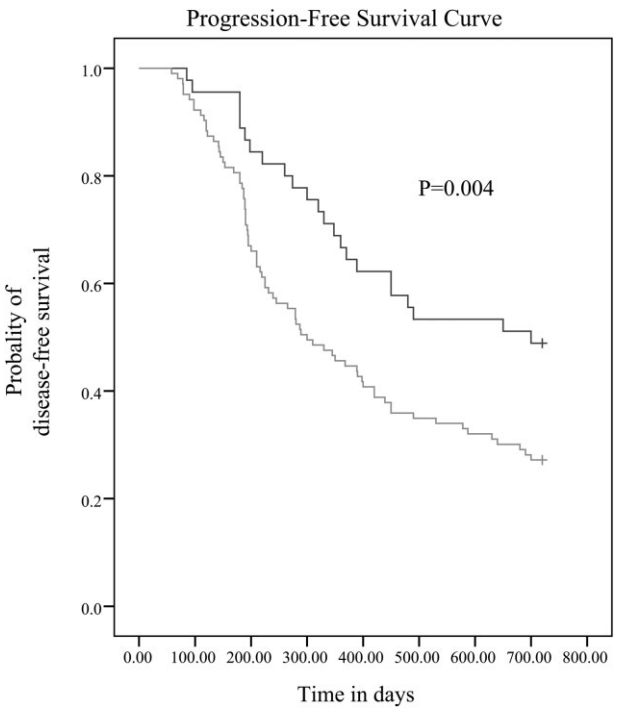

Figure 1 Two-year disease-free survival curve for the tested and non-tested groups. Treat: ⌐¬, Tested group; ⌐¬, Non-tested group; ⊣⊢, Tested group censored; ⊣⊢, Non-tested group censored.

Discussion

The treatment strategy for stage IIIA NSCLC patients is combined therapy based on surgical resection and postoperative chemotherapy as one of the most important adjuvant therapies. Compared with surgery alone, postoperative chemotherapy can be significantly beneficial for patients with respect to DFS. However, chemotherapy itself also has certain limitations; for example, the gradual increase in drug toxicity caused by chemotherapeutic drugs makes patients intolerant to them, while certain tumors can have intrinsic resistance to chemotherapy drugs, which often leads to treatment failure. In recent years, several investigations have shown that there are correlations between the differential gene expression of chemotherapeutic drug targets and the susceptibility of tumors to clinical treatment.[15–17] Theoretically, personalized adjuvant chemotherapy based on molecular tests of these genes (e.g. by quantitative fluorescent PCR) could improve response rates and clinical outcomes.

Researchers have recently evaluated a personalized chemotherapy strategy based on the expression level of the genes associated with drug sensitivity; however, the results have been inconsistent. Several studies have focused on the evaluation of tailored chemotherapy based on tumoral ERCC1 or RRM1 expression levels in advanced NSCLC patients. The research demonstrated the clinical feasibility of an ERCC1/RRM1 gene expression-based chemotherapy regimen selection and revealed a one-year survival rate in the genetically

Table 5 Stratified comparison of one and two-year disease-free survival rates between the tested and non-tested groups

	Disease-free survival	Tested group	Non-tested group	X^2	*P*
T3N1M0	One-year	0.762 (16/21)	0.491 (28/57)	4.573	0.032
	Two-year	0.571 (12/21)	0.333 (19/57)	5.178	0.023
T3N2M0	One-year	0.563 (9/16)	0.393 (11/28)	1.182	0.277
	Two-year	0.313 (5/16)	0.179 (5/28)	1.040	0.308
T1-2N2M0	One-year	0.625 (5/8)	0.389 (7/18)	1.242	0.265
	Two-year	0.500 (4/8)	0.222 (4/18)	2.006	0.157
T1-3N2M0	One-year	0.583 (14/24)	0.391(18/46)	2.344	0.126
	Two-year	0.375 (9/24)	0.196 (9/46)	2.656	0.103

Table 6 Stratified comparison of one and two-year overall survival between the tested and non-tested groups

	Overall survival	Tested group	Non-tested group	X^2	*P*
T3N1M0	One-year	0.905 (19/21)	0.877 (50/57)	0.114	0.735
	Two-year	0.810 (17/21)	0.649 (37/57)	1.854	0.173
T3N2M0	One-year	0.750 (12/16)	0.643 (18/28)	1.035	0.309
	Two-year	0.563 (9/16)	0.536 (15/28)	0.029	0.864
T1-2N2M0	One-year	0.875 (7/8)	0.833 (15/18)	0.074	0.789
	Two-year	0.625 (5/8)	0.611 (11/18)	0.005	0.946
T1-3N2M0	One-year	0.791 (19/24)	0.717 (33/46)	0.455	0.5
	Two-year	0.583 (14/24)	0.565 (26/46)	0.21	0.884

tested group of patients of 59%, significantly better than in the non-tested group.[18,19] Further research confirmed that genetically tested tailored chemotherapy improved OS and DFS compared with the conventional treatment selection approach.[9] However, the latest randomized phase III clinical trial results demonstrated that tailored chemotherapy based on ERCC1/RRM1 protein expression levels did not significantly benefit NSCLC patients with respect to OS and DFS.[20] The differences between these two studies could be a result of differences during patient selection and the chemotherapy regimens used. The fact these studies only detected ERCC1/RRM1 and did not include other chemotherapy drug resistance genes may be another limitation affecting the results. Nonetheless, subgroup analysis in the latter study showed that patients with low levels of both ERCC1 and RRM1 expression had significantly better DFS when treated with the standard doublet therapy of gemcitabine and carboplatin.

The current common chemotherapy drugs are vincristine, paclitaxel, gemcitabine, pemetrexed, and cisplatin. Several studies have suggested that high levels of ERCC1 gene expression are associated with cisplatin resistance and that high levels of RRM1 gene expression are associated with gemcitabine resistance. Moreover, high levels of TUBB3 gene expression are associated with resistance to vincristine and paclitaxel, while high levels of TYMS gene expression are associated with resistance to pemetrexed. Therefore, it is not sufficient to select the chemotherapy regimen based only on levels of ERCC1 and RRM1 gene expression; more comprehensive testing should yield more accurate predictions. A recent study examined ERCC1, RRM1, and TUBB3 gene expression levels in NSCLC patients, and the results showed that the genetically tested group of patients had better DFS and OS than the non-tested group.[21]

We performed a retrospective analysis to evaluate whether the above method could improve the OS and DFS of stage IIIA NSCLC postoperative patients. Kaplan–Meier curve analysis showed that the tested group had a much better two-year PFS than the non-tested group, and the difference was statistically significant ($X^2 = 8.228$, $P = 0.004$). The positive results obtained in this study were attributed to the following reasons: first, the patients enrolled in this study were stage IIIA patients, and second, the four chemotherapy drug-associated genes were all tested. However, the follow-up time in this study was only two years, and longer survival data are lacking.

The results of this study showed that both the one and two-year DFS rates in the tested group were much higher than those in the non-tested group. The differences between the two groups in the one and two-year DFS rates were 22.1% and 21.7%, which is a greater difference than that reported previously.[9,21] The improved DFS rate could be a result of the earlier disease stage of the patients in our study, and, particularly, the fact that they were undergoing surgery. Further stratified analysis showed that the tested group had significantly higher one and two-year DFS rates compared with the non-tested group in T3N1M0 patients. However, in T3N2M0 and T1-2N2M0 patients, although the tested group had a higher two-year DFS than the non-tested group, the difference was not significant. This result could be because of the small sample size of the N2 patients and the short follow-up time. With respect to the adverse effects of the selected chemotherapy regimens, we did not observe serious adverse events (III-IV) in any of the patients. There was no significant difference between the tested and non-tested groups. This observation is consistent with other studies.[21]

Conclusion

Our study demonstrated that a chemotherapy selection method based on the tumoral mRNA expression levels of genes associated with drug sensitivity is clinically feasible and effective. This treatment strategy significantly benefited stage IIIA NSCLC patients, resulting in longer DFS rates without increasing the adverse effects of chemotherapy. Further prospective studies that enroll more patients with longer follow-up times are needed to confirm the clinical efficacy of this chemotherapy selection method. Currently, although no definitive conclusion can be drawn that this method benefits patients, resulting in better survival rates, our results demonstrated that this method does not increase the toxicity of chemotherapy. Considering the potential benefit of this method,

tailored chemotherapy is one of the most important avenues for personalized medicine in lung cancer care.

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Disclosure

No authors report any conflict of interest.

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