RESEARCH ARTICLE

mRNA Expression and Clinical Significance of ERCC1, BRCA1, RRM1, TYMS and TUBB3 in Postoperative Patients with Non-Small Cell Lung Cancer

Yi Han[1&], Xiao-Bin Wang[2&], Ning Xiao[1], Zhi-Dong Liu[1*]

Abstract

Background: To explore mRNA expression and clinical significance of ERCC1, BRCA1, RRM1, TYMS and TUBB3 genes in tumor tissue of postoperative patients with non-small cell lung cancer (NSCLC). Materials and Methods: Sixty NSCLC patients undergoing radical operation in our hospital from Nov., 2011 to Jun., 2012 were selected. Plasmid standards of ERCC1, BRCA1, RRM1, TYMS and TUBB3 were established and standard curves were prepared by SYBR fluorescent real-time quantitative PCR analysis. Samples from tumor centers were taken to detect mRNA expression of ERCC1, BRCA1, RRM1, TYMS and TUBB3 genes in cancerous tissue during operation. The total mRNA expression quantities were compared according to different clinical characteristics. Results: The total expression quantities of 5 genotypes from high to low were ERCC1>RRM1>TUBB3>TYMS>BRCA1 in turn. By pairwise comparisons, other differences showed statistical significance ($p<0.05$ or $p<0.01$) except for TYMS and TUBB3 ($p>0.05$); the low expression rates from high to low were ERCC1>TYMS>TUBB3>TUBB3>RRM1>BRCA1 in turn. The expression quantities of BRCA1, RRM1 and TYMS in males, smokers and patients without adenocarcinoma were all significantly higher than that in females, non-smokers and patients with adenocarcinoma, and significant differences were present ($p<0.05$ or $p<0.01$). In terms of pathological staging, the expression quantities of BRCA1, RRM1 and TYMS in phases IIa~IIb and IIIa~IIIb had a tendency to be greater than in phases I and IV. Conclusions: Resistance to chemotherapy and sensitivity to targeted therapy differ among patients with NSCLC. Differences in gene expression in different individuals were also revealed. Only according to personalized detection results can individualized therapeutic regimens be worked out, which is a new direction for oncotherapy.

Keywords: Non-small cell lung cancer - ERCC1- BRCA1 - RRM1 - TYMS - TUBB3 - mRNA

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Introduction

With the highest morbidity and mortality, lung cancer is one of the most common malignant tumors in the world, in which about 80% pertains to non-small cell lung cancer (NSCLC). About 75% of patients with NSCLC are at an advanced stage (phase IIIb or IV) on the first visit due to lack of effective diagnostic methods at an early stage, hence, they lose the opportunity of operation, and cannot receive a radical therapy. They only depend on chemoradiotherapy to relieve the pathological condition, improve symptoms and prolong survival time, but 5-year survival rate is less than 15%. Evidence-based medicine evidences confirmed that for the patients with NSCLC at phases I-IIIA, the operation should be as the major therapeutic principle, and adjuvant chemotherapy should be performed after radical resection according to tumor staging (Gautschi et al., 2008; Jazieh et al., 2010). However, the therapeutic effect of adjuvant chemotherapy after NSCLC complete excision is not good enough, which mainly results from the drug resistance of tumor cells to anticarcinogen. In recent years, how to improve patients' survival rate by postoperative adjuvant chemotherapy, and how to select the drugs for postoperative adjuvant chemotherapy to make patients obtain more benefits become a focused topic in the field of lung cancer. Studies revealed that a prospective detection to molecular markers is conductive to formulation of individualized therapeutic regimens and enhancement of chemotherapeutic effects (Bartolucci et al., 2009; Santos et al., 2009; Vilmar et al., 2009). For example, the expression levels of excision repair cross complementing 1 (ERCC1), breast cancer susceptibility gene breast cancer 1 (BRCA1) and ribonucleotide reductase M1 (RRM1) are closely associated with the therapeutic effects of chemotherapy drugs and prognosis, and may become the important factors to predict the therapeutic effects so as to conduct individualized treatment (Zhang et al., 2012). Hence,

*[1]Department of Thoracic Surgery, Beijing Chest Hospital, Capital Medical University, Beijing, [2]Department of Thoracic Cardiovascular Surgery, Inner Mongolia Forestry General Hospital, Yakeshi, China [&]Equal contributors *For correspondence: lzdzrd@sina.com*

Table 1. mRNA Synthetic Expression of ERCC1, BRCA1, RRM1, TYMS and TUBB3 Genes

Genotypes	Total expression quantities	Expression results [n(%)]					
		negative	low	middle	A little bit low	A little bit high	high
ERCC1	0.5661±0.2163	1(1.67)	24(40.00)	11(18.33)	12(20.00)	7(11.67)	5(8.33)
BRCA1	0.0617±0.0700**	21(35.00)	9(15.00)	9(15.00)	4(6.67)	7(11.67)	10(16.67)
RRM1	0.3088±0.1574**$^{\#\#}$	0(0.00)	13(21.67)	11(18.33)	5(8.33)	4(6.67)	27(45.00)
TYMS	0.1889±0.1739**$^{\#\#\triangle\triangle}$	0(0.00)	15(25.00)	14(23.33)	8(13.33)	6(10.00)	17(28.33)
TUBB3	0.2226±0.2583**$^{\#\#\triangle}$	1(1.67)	14(23.33)	10(16.67)	19(31.67)	6(10.00)	10(16.67)

Compared with ERCC1, **p<0.01; compared with BRCA1, $^{\#\#}p$<0.01; compared with RRM1, $^{\triangle}p$<0.05, $^{\triangle\triangle}p$<0.01

Table 2. Relationships between Expression of ERCC1, BRCA1, RRM1, TYMS and TUBB3 and Clinical Characteristics

Clinical characteristics	n	ERCC1 expression quantity	BRCA1 expression quantity	RRM1 expression quantity	TYMS expression quantity	TUBB3 expression quantity
Gender						
Male	35	0.5653±0.2626	0.0852±0.0790*	0.3450±0.1723**	0.2317±0.1913**	0.3450±0.1723
Female	25	0.5673±0.1314	0.0288±0.0357	0.2581±0.1192	0.1290±0.1269	0.2598±0.2883
Smoking						
No smoking	32	0.5517±0.3440	0.0329±0.0403**	0.2778±0.1440*	0.1500±0.1463**	0.2086±0.1812
Smoking	28	0.5826±0.2412	0.0946±0.0821	0.3443±0.1669	0.2335±0.1940	0.2385±0.3280
Histological types						
Adenocarcinoma	35	0.5993±0.1625	0.0430±0.0662**	0.2694±0.1339**	0.1522±0.1599**	0.2896±0.2880**
Non-adenocarcinoma	25	0.5196±0.2715	0.0879±0.0680	0.3639±0.1733	0.2404±0.1827	0.1287±0.1753
Pathological staging						
Phase I	7	0.5751±0.2763	0.0729±0.1242	0.2287±0.1746	0.1443±0.1798	0.1860±0.1673
Phases IIa~IIb	28	0.5574±0.1909	0.0791±0.0690	0.3460±0.1579$^{\#\#}$	0.2477±0.2121$^{\#\#}$	0.2181±0.2906
Phases IIIa~IIIb	17	0.5803±0.2366	0.0530±0.0430	0.2837±0.1599	0.1376±0.0880$^{\triangle\triangle}$	0.2646±0.2797
Phase IV	8	0.5585±0.2435	0.0098±0.0276$^{\#\#\triangle\triangle\blacktriangle\blacktriangle}$	0.3021±0.1201$^{\#\#}$	0.1313±0.0998$^{\triangle\triangle}$	0.1809±0.1617

Compared with respective clinical characteristics, *p<0.05, **p<0.01; compared with phase I, $^{\#\#}p$<0.01; compared with phases IIa~IIb, $^{\triangle\triangle}p$<0.01; compared with phases IIIa~IIIb, $^{\blacktriangle\blacktriangle}p$<0.01

tumor samples from 60 patients with excised NSCLC are given target detection of individualized treatment in the study, including detection of mRNA expression quantities of ERCC1, BRCA1, RRM1 and thymidylate synthetase (TYMS) and human β-tubulin-III (TUBB3) genes. In addition, the relationships between expression quantities of genes above and clinical characteristics are also investigated to guide the early diagnosis of NSCLC and postoperative individualized adjuvant chemotherapy. It is reported as follows.

Materials and Methods

General data

Sixty NSCLC patients undergoing radical operation in our hospital from Nov., 2011 to Jun., 2012 were selected. All patients had measurable tumor focuses, in which males were 35 cases, females 25 cases. They were 31-81 years old, and the mean age was (59.58±10.43) years old; smokers were 28 cases, non-smokers 32 cases; ECOG score in performance status was 0-1; histological types were squamous carcinoma 24 cases, adenocarcinoma 35 cases, adenosquamous carcinoma 1 case; postoperative pathological staging included phase I 7 cases, phase IIa 24 cases, phase IIb 4 cases, phase IIIa 14 cases, phase IIIb 3 cases and phase IV 8 cases.

Methods

Plasmid standards of ERCC1, BRCA1, RRM1, TYMS and TUBB3 were established respectively, and standard curves were prepared by SYBR fluorescent real-time quantitative PCR analysis. The samples in tumor center were taken to detect mRNA expression of ERCC1, BRCA1, RRM1, TYMS and TUBB3 genes in cancerous tissue during operation.

Observation indicators

The total mRNA expression quantities and results of ERCC1, BRCA1, RRM1, TYMS and TUBB3 genes were compared, and the differences among each gene mRNA expression quantity were compared according to different clinical characteristics of patients.

Statistical analysis

SPSS13.0 software was applied to conduct a statistical analysis. mRNA expression quantities of ERCC1, BRCA1, RRM1, TYMS and TUBB3 genes were represented by (mean ± standard deviation). t test was used to compare among groups, and expression results were expressed by percentage; variance test was applied to compare among groups. p<0.05 was represented differences had statistical significance.

Results

mRNA synthetic expression of each gene

The total expression quantities of 5 genotypes from high to low were ERCC1>RRM1>TUBB3>TYMS>BRCA1 in turn. By pairwise comparisons, other differences showed statistical significance (p<0.05 or p<0.01) except for

TYMS and TUBB3 (p>0.05) (Table 1).

The low expression rates of 5 genotypes from high to low were ERCC1>TYMS>TUBB3>TUBB3>RRM1 >BRCA1 in turn, in which the negative expression rate of BRCA1 (35%) was the highest, other genotypes (only 1.67% or no expression) were lower (Table 1).

Relationships between mRNA expression of each gene and clinical characteristics

The expression quantities of BRCA1, RRM1 and TYMS in males, smokers and patients without adenocarcinoma were all significantly higher than that in females, non-smokers and patients with adenocarcinoma, and significant differences were presented (p<0.05 or p<0.01), whereas there was no statistical significance between the expression quantities of ERCC1 and TUBB3. In terms of pathological staging, significant differences were not presented regarding the expression quantities of ERCC1 and TUBB3 among each staging (p>0.05), whereas the expression quantities of BRCA1, RRM1 and TYMS at phases IIa~IIb and IIIa~IIIb had a tendency of exceeding those at phases I and IV (Table 2).

Discussion

Clinical practice revealed that the difference of chemotherapeutic effects among individuals is greater in process of oncotherapy, from which only a few patients obtain benefits. Their effective rate is only 20%-40%, medial survival time 8-10 months and a 5-year survival rate less than 15%. However, there are also a few patients without any improvement after treatment, and also undergo the injury caused by adverse reactions to the body. Theoretically, postoperative adjuvant chemotherapy can control or remove the epibiotic micrometastasis after operation for lung cancer to a certain extent, consequently leading to enhancement of long-term survival rate. Due to a low effective rate of chemotherapy, some problems begin to emerge, such as which patients are suitable for adjuvant chemotherapy and how to select postoperative adjuvant chemotherapy to improve postoperative 5-year survival rate better. Hence, an individual or crowd capable of obtaining benefits most from chemotherapy is screened out by detecting biological indicators like genes and proteins, which is of great importance to realize NSCLC individualized treatment, improve individual therapeutic effects and decrease toxic and side effects. Detecting the expression levels of RRM1, ERCC1 and BRCA1 genes in tumor tissue to screen out the patients sensitive to capecitabine is a hot topic in the field of lung cancer at present (Lee et al., 2008; Koh et al., 2010; Hubner et al., 2011).

Nucleotide excision repair (NER), a serious of enzymes exerting an important effects in the process of damaged DNA repair, is considered to involve in the repair after DNA damage induced by chemotherapy drugs. ERCC1 and RRM1 are two important members in NER (Li et al., 2013). ERCC1 positioned on number 19 chromosome, is one of the crucial members in NER family. By encoding proteins of 297 amino acids and forming a heterodimer with XPF, it shears in 5' terminal of single-strand damaged DNA to exert an effect, and its expression directly affects the whole process of DNA repair. Over-expression of ERCC1 can make the damaged DNA in cells stagnating at phase G2/M repair quickly, resulting in the resistance to cisplatine. However, detection of mRNA expression of ERCC1 gene can significantly improve effective and survival rates of patients with tumor before platinum chemotherapy. RRM1 is a subunit of ribonucleotide reductase to regulate M1. RRM1 will provide nucleotides to fill in the vacancy after ERCC1 and repair genes like XPD, XPG and XPA excise the damaged part in DNA chain (Li et al., 2011). RRM1 is not only a tumor suppressor gene, but also a major action target of capecitabine.

BRCA1 gene positioned on human 17q21 chromosome contains 24 exons (in which 22 ones have the coding function), and plays an important role in repair of DNA damage in cell cycles. It also participates in NER and homologous recombination repair. Thymidylate synthetase encoded by TYMS genes is a velocity-limiting enzyme synthesized by pyrimidine nucleotide and an important factor of tumor growth. Its high expression is associated with pemetrexed resistance. The patients with low level of TYMA mRNA have better therapeutic effects after receiving pemetrexed chemotherapy, and the medial survival time is longer. On the contrary, those with high expression have worse therapeutic effects. Tubulin-III (type 3 microtubulin) encoded by TUBB3 has the closest association with the sensitivity of anti-microtubulin chemotherapy drugs. The tumor patients with low expression of TUBB3 have better therapeutic effects for receiving taxanes, and the medial survival time is longer, while those with high expression of TUBB3 have worse anti-microtubulin chemotherapy effects.

In the study, mRNA expression quantities of ERCC1, BRCA1, RRM1, TYMS and TUBB360 genes in tumor tissue of postoperative patients with NSCLC were detected, and the relationships between expression quantities of genes above and clinical characteristics were also investigated. The results revealed that the total expression quantities of 5 genotypes from high to low were ERCC1>RRM1>TUBB3>TYMS>BRCA1 in turn, whereas the low expression rates of 5 genotypes from high to low were ERCC1>TYMS>TUBB3>TUBB3>RRM1 >BRCA1 in turn. The expression quantities of BRCA1, RRM1 and TYMS in males, smokers and patients without adenocarcinoma were all significantly higher than that in females, non-smokers and patients with adenocarcinoma. In terms of pathological staging, the expression quantities of BRCA1, RRM1 and TYMS at phases IIa~IIb and IIIa~IIIb had a tendency of exceeding those at phases I and IV. These results preliminarily revealed the relationship between clinical characteristics of patients with NSCLC and relevant mRNA expression of genes.

Analysis on mRNA expression results of ERCC1 gene demonstrated that 40.00% pertained to low expression in postoperative patients with NSCLC, suggesting that 40.00% of patients with NSCLC are sensitive to platinum chemotherapy, but the resistance to drugs is not dependent on the expression level of one gene or protein at all (Cobo et al., 2007). On the other hand, the detection of expression

quantity to this gene can preliminarily screen out a part of patients with drug resistance so as to guide clinical administration better. Later, combination with other targets may need to be combined to judge.

It can be seen from comparison on mRNA expression of TUBB3 and RRM1 genes, the level of TUBB3 expression was lower than that of RRM1, suggesting that the drug resistance to docetaxel chemotherapy may be relatively lower than that to capecitabine (Maus et al., 2013). The comparison on mRNA expression of TYMS gene demonstrated that the patients with adenocarcinoma were significantly lower than those without adenocarcinoma, and TYMS gene in 25.00% of patients had low mRNA expression, illustrating that pemetrexed has advantages in chemotherapy of patients with lung cancer (Dimoudis et al., 2012). Therefore, adenocarcinoma patients with high mRNA expression of TUBB3 and RRM1 genes can select the chemotherapy of pemetrexed in combination with cisplatin.

A lot of studies revealed that research on single gene cannot judge the prognosis accurately, whereas joint detection of genes can enhance the predictive accuracy (Vilmar et al., 2010; Leng et al., 2012; Pesta et al., 2012). At present, more prospective randomized clinical studies need to be done to select an appropriate molecular genetic indicator combination, consequently providing important references for the formulation of individualized chemotherapy regimens of patients with NSCLC. By detecting mRNA expression of multiple genes in postoperative patients with NSCLC, it is found in the study that the resistance to chemotherapy drugs and sensitivity to targeted therapy are different among different types of patients with NSCLC. The differences of gene expression in different individuals are also revealed even though the patients pertain to the same type of NSCLC. Only according to personalized detection results can individualized therapeutic regimens be worked out, which is a new direction for oncotherapy.

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