Expression of ERCC1 mRNA in Non-small Cell Lung Cancer Tissues and Survival Analysis of Patients

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ABSTRACT: Objective In order to improve the quality of life for non-small cell lung cancer (NSCLC) patients, this study investigated excision repair cross complementing 1(ERCC1) mRNA expression levels in NSCLC and analyzed the factors affecting patient survival after operation. **Methods** ERCC1 mRNA expression levels were examined in 60 cases of NSCLC tissues and adjacent normal tissues by using quantitative Real-Time reverse transcription polymerase chain reaction (RT-PCR) technology. The Kaplan Meier technique and Cox regression were used for survival analysis of patients. **Results** There was a statistical difference between cancer tissues and adjacent normal ones at the mRNA level for ERCC1 (-7.85±3.86, -11.19±5.03, t=3.973, P=0.000). Survival analysis through the Kaplan-Meier method showed the survival rate of patients with high ERCC1 mRNA expression was lower than that of patients with low ERCC1 mRNA expression (P<0.05); Cox regression survival analysis showed that the expression of ERCC1 mRNA, lymph node metastasis, smoking history, pathological grade were significantly independent risk factors affecting survival. The relative risks were 46.698 (95% CI 3.007~125.236), 2.266 (95% CI 1.160~4.428), 1.906(95% CI 1.057~3.437) and 1.339(95% CI 1.114~1.910), respectively (P<0.05). **Conclusions** NSCLC patients with higher ERCC1 mRNA expression have shorter survival time than those with lower ERCC1 mRNA expression. The expression of ERCC1 mRNA, lymph node metastasis, smoking history, family history of cancer and pathological grade can be used as prognostic indicators for NSCLC patients.
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Keywords: ERCC1; Non-small cell lung cancer (NSCLC); Real-time PCR; Survival analysis.

1.INTRODUCTION

Lung cancer is the most common malignant tumor. According to the latest oncoepidemiology data in 2011, lung cancer has ranked first in all malignant tumors on male incidence rate and case-fatality rate up to 2008. It ranks fourth on female incidence rate and second on female case-fatality rate. According to the World Health Organization Statistics, the incidence rate of lung cancer increases gradually in China. Non-small cell lung cancer (NSCLC) accounts for 75%~80% of all lung cancer cases and most cases belong to advanced stage (IIIB and IV stage) when diagnosis. The rate of 5-year survival of advanced lung cancer is less than 5% while the rate of 5-year survival of all lung cancer cases is only 15% [1]. Therefore, better understanding of NSCLC biology pathogenesis and NSCLC prognostic influencing factors has guiding significances for prediction and decision of diseases and choice of treatment.

In recent years, with the development of oncomolecularbiology, it is found that excision repair cross-complementation gene 1 (ERCC1) occupies an important position in maintaining tissue repair function. Whether the DNA loss brought by endogenous cancerogen or exogenous cancerogen, DNA repair genes play an important role in repair damage and the process of maintaining genome stability [2]. Studies show that the expression level of ERCC1 is closely related to chemotherapy drug efficacy and prognosis. Located on No.19 chromosome, ERCC1 is one of the important members of nucleotide exonuclease repair enzyme family and an important member of nucleotide excision repair family. Encoding 297 amino acids, ERCC1 forms heterodimer with XPF and shears on 5' end of DNA single chain damage part to function. Its low expression is often accompanied with increase of lung cancer incidence rate while high expression is always related to platinum preparations and resistant drugs [3, 4]. This study determined the expression levels of ERCC1 mRNA in 60 cases of NSCLC tissues and adjacent normal tissues by using quantitative Real-Time RT-PCR technology and carried out survival analysis combined with The Kaplan Meier technique and Cox regression. We also studied the related factors affecting NSCLC patient survival after operation and investigated prognostic value of expressions of ERCC1 mRNA and others on different patients with

the aim to provide theoretical basis for judging the survival of patients with non-small cell lung cancer after surgery.

2.OBJECTIVES and METHODS

2.1 Objectives

2.1.1 Objective of Study

From March 2005 to June 2006, a total of 60 NSCLC patients with surgical resection and pathological diagnosis from the Thoracic Surgery and Oncology Department of the First Affiliated Hospital of Zhengzhou University were enrolled in this study. All patients did not receive chemicotherapy or other anti-tumor therapies. There were 45 males and 15 females with a mean age of 57.4 years old. Patients' cancer tissues and adjacent normal lung tissues were collected. According to WHO histology grading standards, 17 cases belonged to class I, 18 cases were class II and 18 cases were class III, with all patients owning completed clinical follow-up data (patients received medication-assisted therapy after operation).

2.1.2 Follow-up visit

Postoperative patients were followed to June 2010 and the death time of death case was recorded. Time-to-surgery of patients was seen as the initial event of survival, the end of follow-up time as end point. The time between the initial event and the end point was defined as the survival time of patients. 30 cases died of lung cancer and survival time was (28.04±6.55) months.

2.2 Methods

2.2.1 Instruments and Reagents

Fluorescent quantitation PCR system ((Bio-Rad, Hercules, CA); Trizol reagent (Invitrogen Company); AMV reverse transcription system (Promega Company); Fluorescent quantitation PCR kit (Roche Diagnosis Co., Ltd)

2.2.2 Design and synthesis of β-actin and ERCC1 primers

The specific primers of β-actin and ERCC1 were designed by Primer 5 software (Table 1). Primers were synthesized by Shanghai Biological Engineering Technology Services Limited Company.

Table 1. Primers for ERCC1 and β-actin genes

Gene	Sequence	PCR Product length /bp
β-actin	5′-GATCATTGCTCCTCCTGAGC-3′	101
	5′-ACTCCTGCTTGCTGATCCAC-3′	
ERCC1	5′-CACTTCTCAACTGCCATTC-3′	282
	5′-GTCCATCCGATACACCC-3′	

2.2.3 ERCC1 mRNA Expression

Total RNA was isolated from the two groups of lung tissues with Trizol reagent. RNA was then converted to complementary DNA (cDNA) by means of the reverse transcriptase (RT) reaction. Multiplex PCR was carried out and normalized to β-actin as an internal control. The analysis of each sample was repeated three times. The cDNA was subjected to PCR with the following primer sequences: ERCC1 forward, 5′-CACTTCTCAACTGCCATTC-3′ and ERCC1 reverse, 5′-GTCCATCCGATACACCC-3′, amplifying a 282-bp product. The reaction conditions of β-actin and ERCC1: 95℃ 3min, 95℃ 20s, 58℃/57℃ 20s, then extending to 72℃ for 30s, collecting fluorescence at 86℃/85℃ for 15 s, thirty circulations, at last, extending to 72℃ for 7 min. The amplification results were analyzed with melting curve and agarose gel electrophoresis.

2.2.4 Data Processing [5]

Multiplex Quantitative PCR System software was used to analyze the data. To make the homogenization processing of gene amplification in action systems, β-actin was used as the reference gene and the discrepancy of ERCC1 gene expression, $\triangle Ct=Ct_{ERCC1}-Ct_{\beta\text{-actin}}$. High △Ct value indicated low expression of ERCC1 gene while low △Ct value indicated high expression of ERCC1 gene. The discrepancy of ERCC1 gene expression in oncology group and in the normal group was represented by △△Ct, $\triangle\triangle Ct=\triangle Ct_{ERCC1\ oncology\ group}-\triangle Ct_{ERCC1\ normal\ group}$. The change multiple of ERCC1 gene expression level in oncology group with respect to the normal group was expressed by Z, namely, the relative quantification of mRNA. The comparative threshold method derived by Livak and Schmittgen according to mathematical derivation was adopted, $Z=2^{-\triangle\triangle Ct}$.

2.2.5 Statistical analysis

Statistical analysis was performed with SPSS 12.0 statistical software. The expression level of ERCC1 mRNA in cancer tissues and adjacent lung tissues was analyzed by using paired t test. Univariate survival analysis was completed with Kaplan-Meier method. The multivariate survival analysis of ERCC1 expression and clinical parameters were performed with Cox regression. P value <0.05 was considered to be statistically significant.

3.RESULTS

RT-PCR analysis

From Table 2, compared with adjacent normal tissues the mean value of ERCC1 mRNA expression level in cancer tissues was 10.13, much higher than that in adjacent normal tissues and having statistical significance (t =3.973, P = 0.000). Based on the mean value 10.13, patients were divided into high expression group and low expression group: 71.67% of ERCC1 mRNA high expression rate (43 cases, 10.13~73.52 expression level) and 28.33% of ERCC1 mRNA low expression rate (17 cases, 1.39~10.13 expression level). (Figure 1)

Table 2 Relative expression level of ERCC1 mRNA in NSCLC tissues and adjacent normal tissues

Group	$\overline{\triangle Ct}$±S	$\overline{\triangle\triangle Ct}$ ±S	$2^{-\triangle\triangle Ct}$	*t*	*P*
NSCLC group	7.85±2.86	-3.34±2.86	10.13(1.39~73.52)	3.973	0.000
Normal group	11.19±0.53	0.00±0.53	1.00(0.69~1.44)		

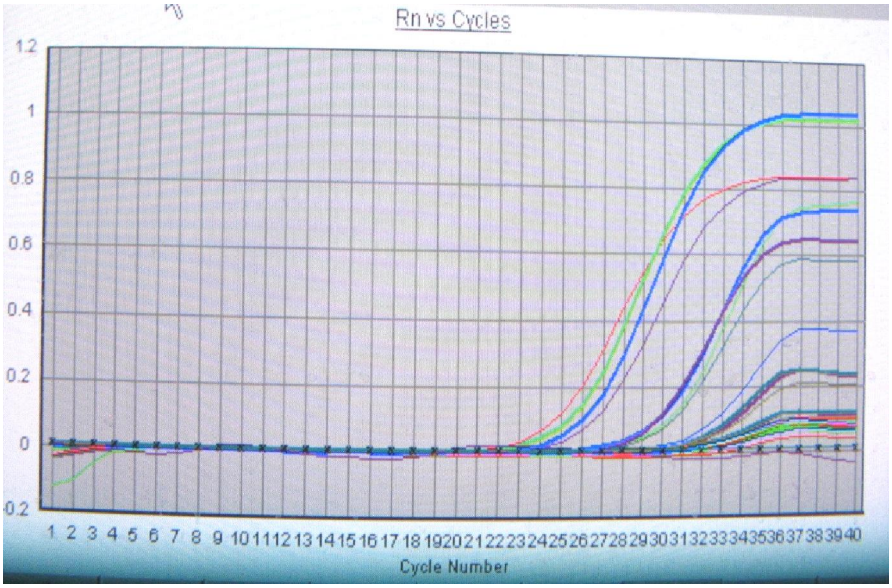

Figure 1 PCR detection power curves of ERCC1 mRNA real-time fluorescent quantitation in NSCLC tissues and adjacent normal tissues

Univariate survival analysis

The patients' survival rate was significantly correlated with the expression level of ERCC1 mRNA, lymph node metastasis, smoking, family history and histological grade. The survival rate was not associated with patient's gender, age, tuberculosis, and tumor volume (Table 3). Figure 2 showed the survival curves of different ERCC1 mRNA expression levels of patients with lymphatic metastasis, smoking, family history and histological grade.

Table 3 The univariate survival analysis results of NSCLC patients

Factor		Total number of cases	Number of death cases	Median Survival/Month	SE	Log-rank	*P*
Gender	Male	45	24	25.33	2.04	0.17	0.6799
	Female	15	6	26. 42	5. 03		
Age	≤50 years	11	4	29. 86	5. 63	0.53	0.7653
	~65 years	34	20	24. 53	2.30		
	≥65 years	15	6	21.31	5. 27		
Lymph node metastasis	Yes	31	22	21.84	2.24	4.97	0.0259*
	No	29	8	31.39	3. 83		
Smoking history	Yes	37	21	19.98	2.36	3. 99	0.0457*
	No	23	9	29.08	3. 41		
Family history of cancer	Yes	12	8	19.17	3.85	3. 89	0.0489*
	No	48	22	26.68	2.36		
Tuberculosis	Yes	9	4	19.98	3.99	0.78	0.3773
	No	51	26	25.68	2.18		
Tumor volume/cm3	≤100	26	9	29.82	3.59	2.37	0.306
	~300	18	10	21.90	3.43		
	≥300	16	11	23.18	3.48		
Pathological grade	I grade	17	5	32.41	4.54	6.94	0.0311*
	II grade	25	9	28.23	3.61		
	III grade	18	16	19.73	2.58		
ERCC1 mRNA	Low expression	17	1	42.47	2.77	12.94	0.0003*
	High expression	43	29	20.99	1.91		

*$P < 0.05$

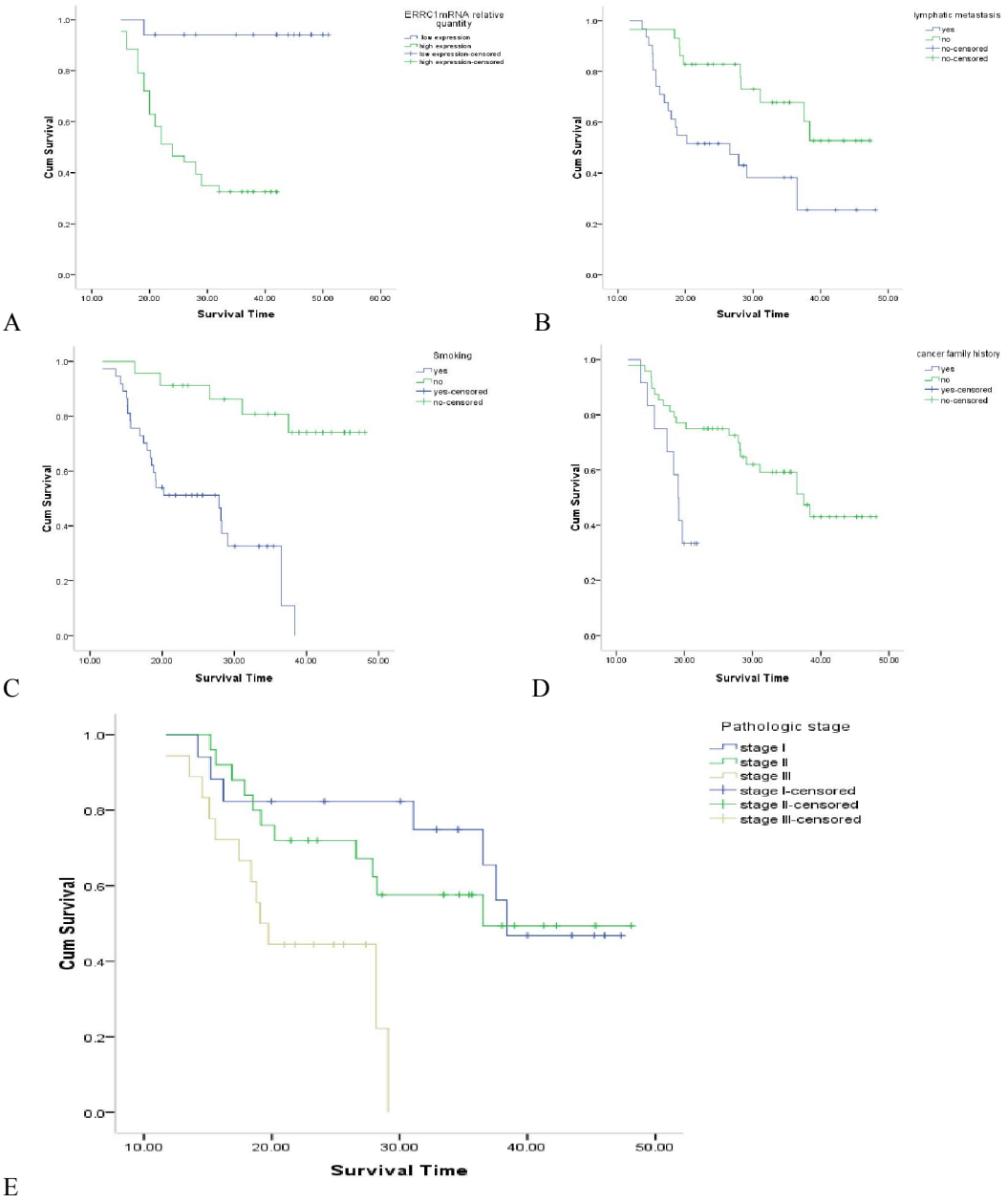

Figure 2 Survival curves of significant influence factors in univariate survival analysis
(A.Survival curves of different mRNA expression level groups of ERCC1genes; B.Survival curves on lymphatic metastasis; C.Survival curves on smoking; D.Survival curves of different family histories; E.Survival curves of different histological grades)

Cox survival analytic results

Significant influencing factors were introduced into COX models. Through multiple analyses, the expression of ERCC1 mRNA, lymph node metastasis, pathological grade and smoking were independent risk factors affecting lung cancer survival(P<0.05). The relative risks were 46.698 (95% CI 3.007~125.236), 2.266 (95% CI 1.160~4.428), 1.906(95% CI 1.057~3.437) and 1.339(95% CI 1.114~1.910), respectively.

Table 4 Multivariate Cox regression analysis of postoperative survival factor of lung cancer patients

Variable	Coefficient (β)	Relative risk	95%*CI*	*Wald*	*P*
ERCC1 mRAN	13.729	46.698	3.007～125.236	7.544	0.006*
lymph node metastasis	0.8516	2.266	1.160～4.428	5.730	0.017*
pathological grade	0.7641	1.906	1.057～3.437	4.592	0.032*
smoking	0.5911	1.339	1.114～1.910	3.773	0.049*
family history	-0.794	0.524	0.162～0.918	2.296	0.130

*$P < 0.05$

4.DISCUSSION

Lung cancer has become the No.1 killer among many tumors around the world and NSCLC has accounted for about 85% of lung cancer. Although multidisciplinary combined treatment method has been applied in NSCLC treatment and improved the therapeutic efficacy, such as molecule target drugs, antibody combined with chemotherapeutics and so on, the survival rate index reflecting patient prognosis situation is still not optimistic. Therefore, understanding NSCLC prognostic influencing factors has a guiding significance for disease prediction, judgment and choice of treatment methods. Studies have found that many clinical pathological factors are related to NSCLC prognostics, for example, pathological grade, lymph node metastasis etc, which become the independent prognostic factors of NSCLC. Besides, gender, smoking, age, weight and other factors may also affect the survival rate of patients [6].

A large number of DNA repair genes exist in the cells. They have specific DNA repair capacity for many types of DNA damage, which play an irreplaceable role in avoiding mutation and disease and maintaining genome stability. ERCC1 is one of the important members in the DNA repairing gene family, which plays a key role in DNA repairing pathways. This study adopted fluorescent quantitation PCR to detect the ERCC1 expression levels in NSCLC tissues and adjacent normal tissues and the results showed that the ERCC1 expression level in NSCLC tissues was higher than that in adjacent normal tissues, which illustrated that ERCC1 played a certain role in the process of NSCLC development.

Numerous studies indicated that low expression of ERCC1 was usually accompanied by the increase of lung cancer incidence, and its high expression often results in patients' resistance to platinum agents [3, 4]. Olaussen et al [7] found that after platinum drug adjuvant therapy, the survival rate of patients with ERCC1 negative expression in NSCLC tissues was higher than that of patients with ERCC1 positive expression in NSCLC tissues. Univariate survival analysis showed that the survival rate of patients with high ERCC1 expression in tissues was much lower than that of patients with low ERCC1 expression, which might be related to postoperative chemotherapy received by patients. It also showed that the survival rate of patients without lymphatic metastasis was much higher than that of patients with lymphatic metastasis, which was consistence with previous studies [8] and indicated that lymphatic metastasis is an important factor affecting prognosis. The survival rate of patients without family history was much higher than that of patients with family history, and the survival rate of patients with high degree of tumor differentiation was much higher than that of patients with low degree of tumor differentiation, which indicated that the degree of malignancy could be regarded as an index determining NSCLC patient postoperative survival and prognosis. This was consistent with the study results of Wisnivesky et al [9].

Some research showed [10] that the prognosis of non-smoking lung cancer was better than that of smoking lung cancer for lung cancer with same staging, for example, the five-year survival rates of I stage were 75% and 62%, respectively (P=0.02), those of II stage were 53% and 46%, respectively (P=0.09), and those of III stage were 41% and 36%, respectively (P=0.13). The prognosis was worse for patients with smoking history over 20 packs of cigarettes per year. Apparently, non-smoking lung cancer was a kind of lung cancer distinct from smoking lung cancer and owning unique biological behavior. The results of this study showed that the survival rate of patients with smoking history was much lower than that of patients without smoking history and it was consistent with previous study results. Researches of Songwen Zhou et al [11] indicated that cancer differentiation grade, age, resection range and adjuvant chemotherapy were independent prognosis risk factors for patients, while our results showed that age was not related to survival rate. Another study reported by Tianshu Liu et al [12] displayed that there was no statistical significance of survival differences between the old group and the young group, which was consistent with our results. CHEN Fang et al [13] made Meta analysis of gender and NSCLC prognosis, showing that gender was an important factor of NSCLC prognosis and the one-year and five-year survival rates of females surpassed those of males. The results of our study showed that gender was unrelated to prognosis survival time, which may

be related to the small amount of selected study samples.

Cox regression analysis results showed that ERCC1 mRNA expression level, lymph node metastasis, pathological grade and smoking were the significant factors which affected the survival rate of NSCLC patients. These factors all can be considered as the indicators to predict prognosis for patients after surgery.

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