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Original Article

Application of gene polymorphisms to predict the sensitivity of patients with locally advanced non-small cell lung cancer undergoing chemoradiotherapy

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Abstract: Objective: To analyze the value of gene polymorphisms in predicting the sensitivity of patients with locally advanced non-small-cell lung cancer (NSCLC) undergoing chemoradiotherapy. Methods: Patients with locally advanced NSCLC undergoing chemoradiotherapy in our hospital from February 2017 to August 2019 were enrolled. X-ray repair cross complementing group 1 (XRCC1) gene polymorphisms were detected before chemoradiotherapy, and the correlation of XRCC1 gene polymorphisms with the sensitivity was analyzed. Results: There was no significant correlation of XRCC1 gene polymorphisms with age, gender, smoking status, pathological type, clinical stage and tumor size ($P > 0.05$). Among 98 patients with locally advanced NSCLC, 17 patients had complete response (CR), 25 patients had partial response (PR), 37 patients had stable disease (SD), and 19 patients had progressive disease (PD). A total of 42 patients were sensitive to chemoradiotherapy (CR + PR), and 56 patients were insensitive to treatment (SD + PD). The effect of XRCC1 gene polymorphisms on the efficacy of chemoradiotherapy was statistically significant ($P < 0.05$). In the codominant model GG vs. GA vs. AA, there was a significant difference ($\chi^2 = 6.473$, $P = 0.039$); The difference between AA and GA was significant ($\chi^2 = 4.572$, $P = 0.032$). The difference between AA and GG was significant ($\chi^2 = 6.003$, $P = 0.014$). There was no significant difference between GA and GG ($\chi^2 = 0.015$, $P = 0.901$). The rates of effective treatment for patients with XRCCI GG vs. GA vs. AA genotypes were 79.17%, 57.14%, and 47.37%, respectively. GG type was 1.38 times more effective than GA type, and GA type was 1.21 times more than AA type. Conclusion: The Arg399Gln polymorphism of XRCC1 gene was significantly related to the sensitivity of patients with locally advanced NSCLC undergoing chemoradiotherapy. The sensitivity of patients carrying wild-type gene AA to chemoradiotherapy was significantly better than that of patients with GA and GG.

Keywords: Gene polymorphisms, non-small cell lung cancer, chemoradiotherapy, sensitivity

Introduction

Lung cancer, the most common malignant tumor worldwide, is classified as small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), with the latter accounting for 80-85% of all lung cancers [1]. Surgery is the mainstay of treatment for early NSCLC. But, upon clinical diagnosis and treatment, the majority of patients were diagnosed at middle and advanced tumor stages and lost the best opportunity for surgical resection [2]. Concurrent chemoradiotherapy is recommended as the first-line therapy for unresectable locally advanced NSCLC, whereas the 5-year survival rate of patients with locally advanced NSCLC has still been extremely poor even with such positive treatment [3]. It has been confirmed that gene mutation is an important factor affecting chemoradiotherapy in cancer patients [4, 5]. The glutamate metabolic pathway can reduce the effect of chemoradiotherapy, enhance the capacity of cellular DNA to repair, and improve the tolerance to cellular DNA damage [6]. Human X-ray cross-complementing gene 1 (XRCC1), as the first isolated gene involved in the repair of mammalian ion radiation damage, has played an important role in the nucleotide excision repair (NER) and base excision repair (BER) pathways, respectively [7, 8]. XRCC1 gene polymorphisms may affect the sensitivity of tumor cells to chemoradiotherapy. It is still unknown whether XRCC1 gene polymorphisms can be used to predict the sensitivity of patients with locally advanced NSCLC to chemoradiotherapy. As a consequence, the sites of XRCC1 gene

Table 1. General clinical information of patients (n = 98)

Clinical characteristic	n (%)
Age (years)	
≥ 60	53 (54.08)
< 60	45 (45.92)
Sex	
M	58 (59.18)
F	40 (40.82)
Smoking status	
Yes	29 (29.59)
No	69 (70.41)
Pathological type	
Adenocarcinoma	63 (64.29)
Squamous cell carcinoma	35 (35.71)
Clinical stages	
Stage III	62 (63.27)
Stage IV	36 (36.73)
Tumor diameter (cm)	
> 7	47 (47.96)
≤ 7	51 (52.04)

polymorphisms were investigated in the current study to predict the sensitivity of patients with locally advanced NSCLC to chemoradiotherapy so as to provide a reference for future studies.

Materials and methods

General data

Patients with locally advanced NSCLC who received chemoradiotherapy in our hospital from February 2017 to August 2019 were selected as the objects of study. Inclusion criteria: (1) patients with complete clinical data; (2) patients who were histopathologically diagnosed as having NSCLC; (3) patients with chemotherapy regimen: consolidation chemotherapy for more than 2 cycles after platinum-based two-drug combination and concurrent chemoradiotherapy, using three-dimensional conformal radiotherapy or intensity-modulated radiotherapy; (4) patients with Karnofsky performance status (KPS) ≥ 70 points; (5) patients used CT and other imaging examination to evaluate the efficacy before and after treatment. Exclusion criteria: (1) patients with severe liver and kidney dysfunction; (2) patients with severe heart, brain and pulmonary vascular diseases; (3) patients with the absence of serious chronic diseases that may affect the timely completion of treatment plan; (4) patients with the presence of distant metastases and other medical or surgical conditions during the treatment period that prevented the completion of the treatment plan as scheduled. A total of 98 patients met the above inclusion and exclusion criteria, and their general information was shown in **Table 1**. This study had been approved by the Ethics Committee of our hospital, and all patients signed informed consent forms.

Methods

Treatment methods: Concurrent or sequential chemoradiotherapy was used. As a three-dimensional conformal or intensity-modulated radiotherapy, it was delivered in 1.8- to 2.0-Gy daily fractions for a total dose of 60-65 Gy (5 times/week). Linear accelerator with 6 MV X-ray involved field irradiation was used, and CT simulation was performed to locate and determine the target area in combination with imaging examination. Chemotherapy regimens included cisplatin plus vinorelbine tartrate (NP), cisplatin plus paclitaxel (TP), cisplatin plus pemetrexed disodium (PP), or cisplatin plus gemcitabine hydrochloride (GP). PP regimen was preferred for adenocarcinoma, and GP regimen was preferred for squamous cell carcinoma, with 4 cycles of chemotherapy in total.

Genetic testing: The XRCC1 gene sequence was: F: 5'-TGTTCTTCCCCTGTGTCTTCGTTCC-3'; R: 5'-ACTTTGTTCTCCCCACCTCCTGA-3'.

The PCR reaction conditions were: 95°C predenaturation for 5 minutes, 95°C 30 s→56°C 30s→72°C 45 s, 35 cycles, and finally 72°C extension for 10 minutes. A total of 5 ml of venous blood were collected from patients before chemoradiotherapy. Genomic DNA was extracted from the specimens using the Maxwell kit, and the DNA concentration was measured using a Nanodrop1000 spectrophotometer, and it was determined that DNA concentrations of all patients met the requirement. Blood samples were detected for XRCC1 gene polymorphisms using SurPle® x-Xtag70plex liquid chip technology. Specific steps were as follows: ① the polymerase chain reaction (PCR) was used to obtain the gene fragments containing common allelic types on the exons of each gene; ② exonuclease digestion and alkaline phosphatase hydrolysis were used to remove excess primers and dNTP; ③ allele-specific primer extension (ASPE) reaction, the specific sequence on primers could specifically

Table 2. HWE test of the gene frequencies of XRCC1 site in patients with locally advanced NSCLC

Site	Genotype	Observed value	Theoretical value	χ^2	*P*
Arg399Gln	GG	52 (53.06)	59.7	3.081	0.063
	GA	29 (29.59)	35.2		
	AA	17 (17.35)	20.6		

recognize each allelic type and form extension products; ④ the Tag sequence on ASPE primers could specifically bind to Anti-Tag sequence on polystyrene microspheres to complete the hybridization reaction; ⑤ the hybridized microspheres were analyzed by Luminex 200 system (multi-functional flow lattice instrument), and the median fluorescent intensity (MFI) of each sample was read. Blank, wild-type control, and negative and positive test results of each mutation were set as references in each experiment to judge the negative and positive for samples. The relationship between polymorphisms and chemotherapy sensitivity was detected.

Efficacy evaluation: After chemoradiotherapy, CT and other imaging examinations were performed to evaluate the therapeutic effects. According to the different degrees of response, these were divided into complete response (CR, disappearance of all lesions for the duration of 4 weeks), partial response (PR, reduced by 30% in the duration of 4 weeks), stable disease (SD) and progressive disease (PD, increased in lesion by 20%).

Statistical methods

SPSS 17.0 software was used to process the data of this study. The enumeration data were expressed as percentage (%), and analyzed by χ^2 test; the quantitative data were expressed as ($\overline{x} \pm s$), and analyzed by t-test; and the gene frequencies at the site were analyzed for genetic balance by Hardy-Weinberg equilibrium (HWE) test. $P < 0.05$ was considered significant. GraphPad prism 8 software was used to plot graphics.

Results

HWE test of the gene frequencies of XRCC1 site in patients with locally advanced NSCLC

The genotype test of the G/A site of XRCC1 G1301A in patients with locally advanced NSCLC by HWE's law showed that there was no significant difference between the observed value and the theoretical value ($P > 0.05$), suggesting that the subjects in this study were representative of the population (**Table 2**).

Correlation between XRCC1 gene polymorphisms and clinical characteristics of patients

XRCC1 gene polymorphisms were not significantly associated with age, gender, smoking status, pathological type, clinical stage, and tumor size ($P > 0.005$), as shown in **Table 3**.

Correlation between XRCC1 gene polymorphisms and the efficacy of chemoradiotherapy

According to the Response Evaluation Criteria in Solid Tumors (RECISTs), among 98 patients with locally advanced NSCLC, 17 (17.35%) patients had CR, 25 (25.51%) patients had PR, 37 (37.76%) patients had SD and 19 (19.39%) patients had PD. Of the 98 patients, a total of 42 (42.86%) patients were sensitive to chemoradiotherapy (CR + PR), and 56 (57.14%) patients were insensitive to treatment (SD + PD). As shown in **Table 4**, the effect of XRCC1 gene polymorphisms on the efficacy of chemoradiotherapy was significant ($P < 0.05$).

Correlation between XRCC1 gene polymorphisms and the sensitivity to chemoradiotherapy

There was a significant difference between sensitive patients and non-sensitive patients in a codominant model GG vs. GA vs. AA ($\chi^2 = 6.473$, $P = 0.039$); there was a significant difference between AA and GA ($\chi^2 = 4.572$, $P = 0.032$). As shown in **Table 5**, there was significant difference between AA and GG ($\chi^2 = 6.003$, $P = 0.014$); there was no statistical difference between GA and GG ($\chi^2 = 0.015$, $P = 0.901$).

The relationship between the polymorphism and chemotherapy sensitivity

The effective rates of treatment for patients with XRCCI GG vs. GA vs. AA genotype were 79.17%, 57.14%, and 47.37%, respectively.

Table 3. Correlation between XRCC1 gene polymorphisms and clinical characteristics of patients

Clinical characteristic	*n*	GG (n = 52)	GA (n = 29)	AA (n = 17)	χ^2	*P*
Age (years)					1.180	0.554
≥ 60	53	27 (50.94)	18 (33.96)	8 (15.09)		
< 60	45	25 (55.56)	11 (24.44)	9 (20.00)		
Sex					1.002	0.606
M	58	32 (55.17)	15 (25.86)	11 (18.97)		
F	40	20 (50.00)	14 (35.00)	6 (15.00)		
Smoking status					0.596	0.742
Yes	29	17 (58.62)	8 (27.59)	4 (13.79)		
No	69	35 (50.72)	21 (30.43)	13 (18.84)		
Pathological type					0.091	0.956
Adenocarcinoma	63	34 (53.97)	18 (28.57)	11 (17.46)		
Squamous cell carcinoma	35	18 (51.43)	11 (31.43)	6 (17.14)		
Clinical stages					0.208	0.901
Stage III	62	33 (53.23)	19 (30.65)	10 (16.13)		
Stage IV	36	19 (52.78)	10 (27.78)	7 (19.44)		
Tumor size					1.421	0.491
> 7 cm	47	27 (57.45)	14 (29.79)	6 (12.77)		
≤ 7 cm	51	25 (49.02)	15 (29.41)	11 (21.57)		

Table 4. Correlation between XRCC1 gene polymorphisms and efficacy of chemoradiotherapy

Efficacy	*n*	Genotype			χ^2	*P*
		GG	GA	AA		
CR + PR	42	29 (69.05)	8 (19.05)	4 (9.52)	8.401	0.015
SD + PD	56	23 (41.07)	21 (37.50)	12 (21.43)		

Table 5. Correlation between G1301A polymorphisms of XRCC1 gene and sensitivity to chemoradiotherapy

Codominant model	*n*	CR + PR (n = 42)	CR + PR (n = 56)	χ^2	*P*
AA	17	12 (70.59)	5 (29.41)	6.473	0.039
GA	29	11 (37.93)	18 (62.07)		
GG	52	19 (36.54)	33 (63.46)		

Table 6. Correlation between XRRC1 gene polymorphism and chemotherapy sensitivity of platinum-based drugs in lung cancer (n)

	effective	ineffective	OR (95% CI)	P
XRCC1				
GG	38	10	2.52 (1.07-5.86)	0.030
GA	20	15	1.47 (0.54-3.99)	0.045
AA	9	10	1.32 (0.07-20.56)	0.789

The effective rate of GG type is 1.38 times that of GA type and 1.21 times of AA type ($P < 0.05$, **Table 6**).

Discussion

In view of the relatively complex biologic characteristics of locally advanced NSCLC, such patients are more likely to be treated clinically with a combination of radiotherapy and chemotherapy [9]. Radiotherapy can directly break DNA strands and produce free radicals to damage DNA so as to kill tumor cells, and can improve local tumor control and reduce local recurrence of tumors [10, 11]. Radiotherapy combined with chemotherapy has better efficacy than radiotherapy alone. Chemotherapy can inhibit DNA synthesis and transcription through the binding of drugs to intracellular DNA to form DNA adducts [12]. However, tumor microenvironment factors, such as hypoxia and lymphocyte infiltration, will affect the sensitivity of chemoradiotherapy, thereby leading to poor efficacy of chemoradiotherapy.

There is a lack of effective biomarkers for predicting the efficacy of first-line concurrent chemoradiotherapy in patients with locally advanced NSCLC. The human XRCC1 gene is located on the long arm

of chromosome 19, 33 kb in length, with three functional domains, and is involved in DNA base repair and recombination repair of DNA single-strand breaks [13]. There are three nucleotide polymorphism sites on XRCC1, namely Arg194Trp, Arg280His, and Arg399Gln [14-17]. The presence of these polymorphism sites affects the function of the encoded protein, thereby affecting the body's susceptibility to tumors and chemosensitivity. The site 399 is located in the functional domain, indicating that it has a greater impact on DNA repair function. Xu et al. [18] reported that JWA human gastric cancer cells could reverse the resistance to cisplatin through CK2-XRCC1 pathway. Huang et al. [19] reported that gastric cancer patients carrying the XRCC1 399Arg/Arg genotype had a significantly lower recurrence rate and mortality rate due to chemoradiotherapy than those carrying the Gln genotype. Gurubhagavatula et al. [20] reported that the XRCC1 gene polymorphism was a prognostic factor affecting lung cancer patients treated with platinum-based chemotherapy. After 98 patients with locally advanced NSCLC included in this study were treated with chemoradiotherapy, 17 patients achieved CR, 25 patients achieved PR, 37 patients had SD, and 19 patients had PD. After analysis, the effect of XRCC1 gene polymorphisms on the efficacy of chemoradiotherapy was found to be significant, and there was a significant difference in the codominant model GG vs. GA vs. AA between sensitive and non-sensitive patients; there was a significant difference between AA and GA; there was a significant difference between AA and GG; but there was no statistical difference between GA and GG. These results suggested that the efficacy of chemoradiotherapy was better in AA gene carriers than that in other gene carriers. Radiotherapy destroyed single or double strands of DNA in tumor cells by X-rays, causing DNA strand breaks. The XRCC1 gene polymorphisms failed to repair DNA strand breaks, resulting in the inability of DNA to replicate and transcribe normally to cause the loss of unlimited proliferation of cells, thereby increasing radiosensitivity. Chemotherapy drugs caused XRCC1 Arg-399Gln site polymorphisms to alter the coding sequence of amino acid so as to destroy their own protein function, which in turn reduced DNA repair function, resulting in changes in DNA repair function, thereby improving chemosensitivity. The results of this study showed that the effective rates of treatment for patients with XRCCI GG vs. GA vs. AA genotypes were 79.17%, 57.14%, and 47.37%, respectively. The effective rate of GG type was 1.38 times that of GA type, and that of GA type was 1.21 times that of AA type. At present, the preliminary research results of a number of individualized treatments of malignant tumors suggest that the DNA repair gene XRCCI polymorphism is likely to become a molecular signature for predicting the effect of platinum drugs in chemotherapy, laying a certain foundation for guiding individualized medication and improving clinical efficacy.

In summary, the Arg399Gln polymorphisms of the XRCC1 gene is significantly associated with the sensitivity of patients with locally advanced NSCLC to chemoradiotherapy, and patients carrying the wild-type gene AA are significantly more sensitive to chemoradiotherapy than those carrying GA and GG types. However, there are many regulatory DNA damage repairs in the human body, and it is also necessary to further understand the regulation of XRCC1 gene in locally advanced NSCLC so as to provide new ideas for improving the sensitivity to chemoradiotherapy and new targets for locally advanced NSCLC.

Disclosure of conflict of interest

None.

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