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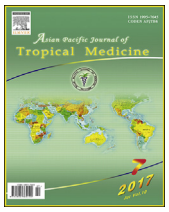

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Association between FGFR4 gene polymorphism and high-risk HPV infection cervical cancer

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ABSTRACT

Objectives: To discuss the association between FGFR4 gene polymorphism rs351855 (Glu388Aly) and the susceptibility and chemotherapeutic effect of cervical cancer infected by high-risk type HPV.

Methods: A total of 162 patients with high-risk HPV cervical cancer and 162 healthy women were collected and the genotypes of the FGFR4 rs351855 locus were detected. The genotype distributions in the two groups were compared. The cervical cancer patients were divided into four groups which namely good therapeutic effect group and bad therapeutic effect, recurrence or metastasis and no recurrence or metastasis group respectively, and the risks of different genotype on the curative effect and prognosis were analyzed by Logistic regression. The survival time of patients with different genotypes was compared.

Results: There was no statistic difference in FGFR4 rs351855 genotype distribution between the patients group and control group ($P > 0.05$), among which the risk of chemotherapy failure on GA + AA patients was 3.257 times as much as that of the GG patients, and the risk of recurrence or metastasis of GA + AA patients was 2.783 times as much as that of the GG patients. For AA patients, the risk of chemotherapy failure and the risk of relapse and metastasis are 3.833 and 3.406 times, respectively, as much as that of the GG patients. The overall survival of GA and AA patients was shorter than that of the GG patients, and significant difference was found ($\chi^2 = 7.098$, $P = 0.029$). The difference in overall survival between GA + AA patients and GG patients was almost statistically significant ($\chi^2 = 3.634$, $P = 0.057$).

Conclusions: The FGFR4 rs351855 polymorphism is not associated with the susceptibility of high-risk HPV cervical cancer, but patients with gene A was at higher risk of unfavorable chemotherapy prognosis compared with patients with GG.

1. Introduction

Cervical cancer was a malignant tumor threatening women's health and lives, whose morbidity and mortality rank only second to the breast cancer among women. Human papilloma virus (HPV), the only confirmed cancer-causing virus so far, was a typically dangerous factor leading to the cervical cancer [1]. According to the degree of risk of causing malignant tumor, it could be divided into the low-risk and high-risk types. The mucosal high-risk type HPV was the primary factor leading to cervical cancer, including HPV16, 18, 26, 31, 33, 35, 45, 51, 52, 56, 58 and 61, *etc*. [2]. Currently, surgery and chemotherapy was the primarily treatments of HPV high-risk type cervical cancer, while significant differences existed in chemosensitivity between different individuals. Based on clinical research, single nucleotide polymorphism (SNP) of related genes was considered a reason causing different chemotherapy effects between individuals [3], therefore clarifying influences of related gene polymorphism on chemotherapy and then customizing

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chemotherapy for individuals was clinically significant. Previous studies have shown that fibroblast growth factor receptor 4 (FGFR4) gene polymorphism was associated with the occurrence, metastasis, and treatment prognosis of many malignant tumors [4,5]. However, there are few researches on association between FGFR4 polymorphism and cervical cancer. This study aimed to discuss the influences of FGFR4 polymorphism on susceptibility of high-risk type HPV cervical cancer and chemotherapy prognosis through analyzing differences in FGFR4 rs351855 (Glu388Aly) genotype distribution between healthy women and high-risk HPV cervical cancer patients as well as chemotherapy effect.

2. Materials and methods

2.1. Cases

Patients with cervical cancer treated in the Xi'an Central Hospital and the First Affiliated Hospital of Xi'an Jiaotong University from January, 2013 to December, 2016. Selection criteria of the cases: (1) Cervical cancer patients infected by high-risk type HPV who were diagnosed by pathology and HC-2; (2) All cases were newly diagnosed and firstly treated, and received platinum-based chemotherapies; (3) All cases were Chinese Han nationality; (4) All cases were informed consent, and accepted related examinations and treatments voluntarily. Patients with a combination of other gynopathy or malignant tumors, or patients in gestation period were excluded. In this study, 162 cases aging 28–65 years old were selected in accordance with the criteria, with the average age of (46.5 ± 9.0) years old. A total of 162 women who have been explicitly diagnosed with no HPV infection in the First Affiliated Hospital of Xi'an Jiaotong University Hospital were concurrently collected as the control group. The control group was aged 22–60 with the average age of (46.00 ± 8.35) years old.

2.2. Grouping for cervical cancer patients

According to the Response Evaluation Criteria in Solid Tumors (RECIST), the curative effect of patients after chemotherapy could be divided in to three levels, namely complete remission (CR), partial remission (PR), and progressive disease (PD). Patients in the case group were kept in touch by telephone or outpatient follow-up and were divided into the recurrence or metastasis group and no recurrence or metastasis group based on recurrence information obtained during the follow-up period. Survival information of each case were collected and recorded.

2.3. Inspection methods

2.3.1. HPV infection testing

Samples were taken from cervical tissue cells of all participants (test for cervical cancer patients were performed before the surgery or any treatments). The sampling brush was rotate in the cervical canals to take samples, and samples were stored in refrigerator at the temperature of 4 °C. The HPV infection status and type in the cervical tissue were tested by HC-2 detector developed by QIAGEN (German), the cervical cancer patients with HPV16, 18, 26, 31, 33, 35, 45, 51, 52, 56, 58 and 61 were selected as case group and the healthy patients with positive HPV were excluded.

2.3.2. FGFR4 genotype detection

Venous blood (5 mL) from all objects was collected to the anticoagulative tube. The peripheral blood DNA was taken by using the kits, and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) were conducted to detect the genotype of FGFR4 rs351855 polymorphic locus. Prime was designed according to reference [6], and synthesized by Beijing SBS gene technology Ltd: Upstream 5′-CTTGG-GTCCCTGAGAGCTGTG-3′ and downstream 5′-CAGAAGC-CATACCAAGCTCCACT-3′. PCR procedures: predegeneration for 5 min at 95 °C, degeneration for 20 s at 95 °C, annealing for 40 s at 65 °C, and extension for 90 s at 72 °C, totaling 35 circulations. At last, extension for 10 min at 72 °C, and the product were stored at 4 °C. The result was tested by 3.0% agarose gel electrophoresis (AGE) after the enzyme digestion.

2.4. Data analysis

All data was analyzed with the IBM SPSS19.0. Hardy–Weinberg genetic balance test was conducted to test the genotype distribution in the case group and control group. Distribution difference in genotype between different groups was compared by using the chi-square test; non-condition Logistic regression was conducted to analyze the odds ratio (OD) of risks and 95%*CI* of FGFR4 genotype in HPV cervical cancer occurring, chemotherapy failure and relapse or metastasis. Survival difference between cervical cancer patients with different genotypes were compared by Kaplan–Meier survival analysis and Log-rank test. $P < 0.05$ was considered statistically significant in all the results.

3. Results

3.1. Association between FGFR4 gene polymorphism and susceptibility of HPV infection cervical cancer

The FGFR4 rs351855 genotype distribution of the case group and control group were shown in Table 1, which was in accordance with the Hardy–Weinberg law of inheritance ($P > 0.05$). It could be seen from Table 1 that there was no statistic difference in FGFR4 Glu388Aly genotype distribution between the case group and control group ($P > 0.05$). The OR value of morbidity risks of GA, AA and GA + AA type were all greater than GG but no statistical significance ($P > 0.05$), which indicated that FGFR4 may be uncorrelated with the susceptibility of HPV infection cervical cancer.

3.2. Association between FGFR4 polymorphism and chemotherapy effect of HPV infection cervical cancer

The FGFR4 Glu388Aly genotype distribution and basic data comparison of the above mentioned 162 high-risk HPV infection cervical cancer patients were shown in Table 2, which showed that there was no statistical significance of differences in the age, stages, pathological type, and treatment method of each patient ($P > 0.05$). The curative effect after chemotherapy treatments and corresponding genotypes were shown in Table 3. CR was considered as good chemotherapy effect while PR + PD as bad effect (chemotherapy failure), then it could be seen from Table 3 that the risk of chemotherapy failure of GA + AA patients was 3 times as much as that of the GG patients, while that of AA

Table 1
Association between FGFR4 rs351855 polymorphism and the susceptibility of high-risk HPV infection cervical cancer [number of cases (proportion)].

Genotype	Control group (162 cases)	Case group (162 cases)	*OR* (95%*CI*)	*P* value	χ^2	*P*
GG	50 (30.9%)	35 (21.6%)	1	0.157	3.699	0.157
GA	72 (44.4%)	79 (48.8)	1.569 (0.917–2.686)	0.100		
AA	40 (24.7%)	48 (29.6%)	1.721 (0.942–3.145)	0.077		
GA + AA	112 (69.1%)	127 (78.4%)	1.623 (0.983–2.680)	0.088	–	–

P value and *OR* value after adjusted for age and other factors.

Table 2
Comparison between baseline information of different genotype in the case group.

Material		*n*	GG (35 cases)	GA (79 cases)	AA (48 cases)	F/χ^2	*P*
Age		–	48.0 ± 11.3	46.1 ± 8.5	46.5 ± 9.5	0.477	0.621
Stage	II	24	5	13	6	1.230	0.873
	III	112	24	52	36		
	IV	26	6	14	6		
Pathological type	Squamous carcinoma	138	28	70	40	1.609	0.447
	Others	24	7	9	8		
Therapy	Postoperative chemotherapy	76	18	35	23	1.077	0.898
	Combined chemoradiotherapy and radiotherapy	50	11	24	15		
	Single chemotherapy	36	6	20	10		

Table 3
Association between FGFR4 rs351855 polymorphism and chemotherapy effect of high-risk HPV infection in cervical cancer [number of cases (proportion)].

Genotype	*n*	CR (99 cases)	PR + PD (63 cases)	*OR* (95%*CI*)	*P*
GG	35	26 (74.3%)	9 (26.7%)	1	0.041
GA	79	47 (59.5%)	32 (40.5%)	2.605 (0.983–6.902)	0.054
AA	48	25 (52.1%)	23 (47.9%)	3.833 (1.344–10.932)	0.012
GA + AA	127	72 (56.7%)	55 (43.3%)	3.257 (1.215–8.142)	0.018

P and *OR* value after adjusted for age, periodization, pathological type, and therapy.

patients was 3.833 times as much as that of GG patients, and all these data was statistically significant ($P < 0.05$).

3.3. Association between FGFR4 polymorphism and relapse or metastasis of HPV infection cervical cancer

Among the 162 high-risk HPV infection cervical cancer patients in this study, 51 patients (31.5%) developed relapse or metastasis after therapy, and the genotype distribution was shown in Table 4. The Logistic regression analysis showed that risk of relapse and metastasis of DA + AA patients was 2.783 times as much as that of the GG type, while that of AA patients was 3.406 times as much as that of the GG patients, which consequently indicated that cervical cancer patients with gene A were at higher risks of relapse and metastasis ($P < 0.05$).

3.4. Association between FGFR4 polymorphism and survival of HPV infection cervical cancer

The result of survival analysis of cervical cancer patients with different genotypes was shown in Figure 1. Logrank test showed that there was statistical difference in survival period between

Table 4
Association between FGFR4 rs351855 polymorphism and relapse and metastasis of high-risk HPV infection in cervical cancer [number of cases (proportion)].

Genotype	*n*	No relapse or metastasis (111 cases)	Relapse or metastasis (51 cases)	*OR* (95%*CI*)	*P*
GG	35	28 (80.0%)	7 (20.0%)	1	0.093
GA	79	54 (68.4%)	25 (31.6%)	2.437 (0.859–6.914)	0.094
AA	48	29 (60.4%)	19 (39.6%)	3.406 (1.127–10.297)	0.030
GA + AA	127	83 (65.4%)	44 (34.6%)	2.783 (1.036–7.480)	0.042

P value and OR value after adjusted for age, periodization, pathological type, and therapy.

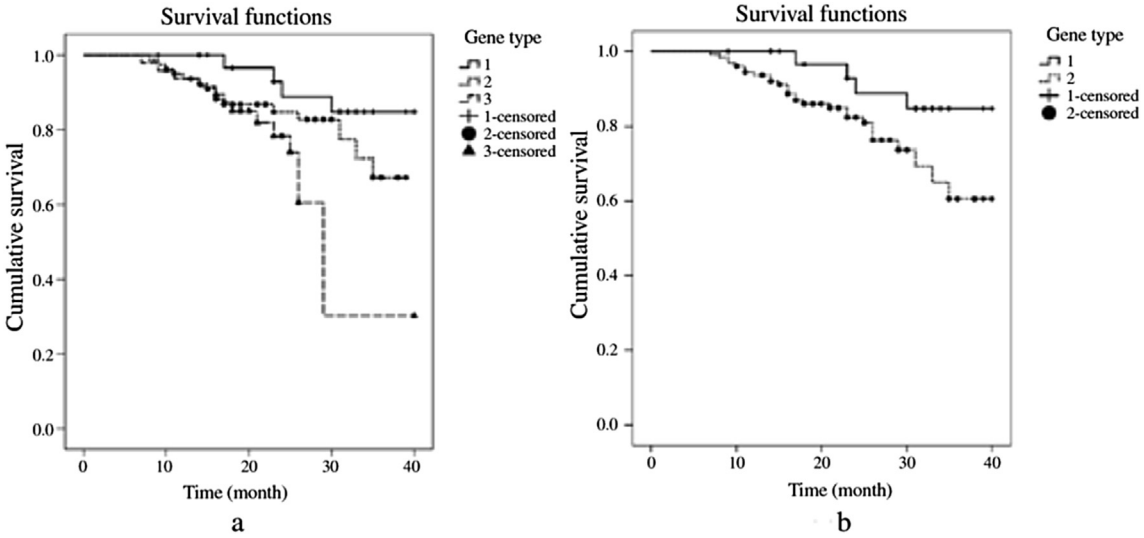

Figure 1. Comparison and analysis of survival function with different genotype.
a: 1 refers to GG type, 2 to GA type, and 3 refers to AA type respectively; b: 1 refers to GG type and 2 to GA + AA type.

GG, GA, and AA patients (χ^2 = 7.098, P = 0.029), and the overall survival of GA and AA patients were shorter than that of GG patients. The survival function of GA + AA patients and GG patients was statistically significant (χ^2 = 3.634, P = 0.057). As such, the overall prognosis of cervical cancer patients with gene A was worse than that of GG patients.

4. Discussion

More than 90% cervical cancer patients were infected with HPV, and most of which were the mucosa high-risk type HPV [7]. FGFR4, is a specific receptor fibroblast growth factor (FGF), together with FGF both playing important roles in proliferation and differentiation of cells. Previous studies had shown that FGFR4 was over-expressed in malignant tumor tissues including cervical cancer [8], and Glu388Arg gene polymorphism influenced the morbidity and treatment of many cancers [9]. However, more studies should be conducted to clarify correlation between FGFR4 polymorphism and cervical cancer especially with the high-risk HPV type infection. In this study, the results of analysis on FGFR4 rs351855 polymorphism of 162 cervical cancer patients indicated that FGFR4 rs351855 polymorphism was uncorrelated with susceptibility of the cervical cancer, but was associated with the chemotherapy prognosis of cervical cancer. Previous studies had shown that FGFR4 Glu388Arg polymorphism was associated with many malignant mucosal tumor such as oral cancer [10], colon cancer [11], esophageal carcinoma [12] *etc*., and affected the morbidity and treatment of many cancers occurred in sexual organs such as prostate cancer [13], ovarian cancer [14], breast cancer [15] and so on. Therefore, it is worthy to deeply explore the risk of FGFR4 Glu388Arg gene polymorphism on causing HPV infection cervical cancer—a mucosal tumor in sexual organs. The latest study of Chen TH (January 2017, Taiwan) [16] indicated that FGFR4 rs351855 polymorphism was associated to the prognosis of cervical cancer, the risk of metastatic lymphadenopathy of patients with gene GA/AA was 3.1 times as much as that of the GG patients and may lead to a shorter survival period. In this study, the result showed that there was no difference in FGFR4 rs351855 genotype distribution between the case group and control group, which meant that it was not associated with susceptibility of the cervical cancer. But patients with A allele were at higher risk of chemotherapy failure, relapse or metastasis and shorter survival period compared with GG patients, which was consistent with the conclusions obtained by Chen TH [16].

FGFR4 was the receptor of FGF and FGF family was one of the most important wound-healing factor families in human body. The two played important repairing effects together when combined on fibroblast mitosis and tissue wound-healing including injury repairing of mucosa [17]. Generally, if a patient was infected with high-risk type HPV, injury in cervical mucosal may be caused firstly and then the cervical cancer could be triggered with the aggravation of the injury. During the process of mucosal injury, the combination of FGF family and FGFR4 played a role in repairing the injury, therefore FGFR4 was at high expression level in cervical cancer tissues. As a transmembrane signaling peptide, FGFR4 could activate the downstream NF-κB signal pathway [18], then promoted proliferation and generation of tumor cells by up-regulating CyclinD1, meanwhile activated downstream signals to promote distant metastasis of tumors [19,20]. FGFR4 rs351855 polymorphism was a mutation from G to A on the 383rd genetic codon, which resulted in the encoded glycine change into arginine and consequently structure variation. The structural change caused by this missense mutation was located in 372–400 transmembrane domain of FGFR4. As a transmembrane signaling peptide, transmembrane domain was so important pathway for transferring signal that the polymorphism was very likely to affect its regulation of downstream factors. Therefore, it could be speculated that FGFR4 rs351855 polymorphism of patients with GA/AA genotype may have higher signal intensity to activate downstream NF-κB pathways and other factors compared with GG patients, which would promote the proliferation and metastasis of cervical cancer cells easier. It meant that the poorer clinical manifestations, including poor curative effect, easy to recurrence, consequently worse prognosis and shorter survival period.

In conclusion, this study showed that FGFR4 Glu388Arg polymorphism was associated with the prognosis of high-risk HPV infection cervical cancer, the risk of worse prognosis in

patients with allele A was higher than that of GG patients, and may consequently influenced the overall survival. The biological mechanism may be the functional difference caused by structural changes corresponding to polymorphism.

Conflicts of interest statement

We declare that we have no conflict of interest.

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