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Original Article

Multiple genetic variants are associated with the development of esophageal squamous carcinoma in the Chinese population

Qijiang Wang[1*], Lifeng Wang[2*], Jiangtao You[3], Hua Yang[4,5], Dongwei He[1], Lun Han[1], Peng Zhang[6], Tianbo Jin[4,5]

[1]2nd Department of General Surgery, Yulin First Hospital, Yulin, Shaanxi, China; [2]Department of General Surgery, Yulin First Hospital, Yulin, Shaanxi, China; [3]Department of Thoracic Surgery, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China; [4]School of Life Sciences, Northwest University, Xi'an, Shaanxi, China; [5]National Engineering Research Center for Miniaturized Detection Systems, Xi'an, Shaanxi, China; [6]Department of Pathology, Yulin First Hospital, Yulin 718000, Shaanxi, China. [*]Equal contributors.

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Abstract: Purpose: To investigate the association between gene factors with the prognosis of esophageal squamous carcinoma (ESCC). Method: One hundred and fifty two ESCC patients were enrolled from 2014 to 2015 in the Yulin First Hospital. Kaplan-Meier survival analysis was performed and cox proportional hazard regression model was used. Results: We investigated 76 SNPs and identified three SNPs (rs12413624, P=0.032; rs9543325, P=0.022; rs225190, P=0.047) were associated with the prognosis of ESCC. The genotype "AT" in rs12413624, "TT" in rs9543325 and "AG" in rs225190 had the longest survival time compared to the other two genotypes. The clinical factors including age and TNM stage were also associated with the prognosis of ESCC. Conclusion: Our study identified three SNPs (rs12413624, rs9543325, rs225190) might be important prognostic factors of ESCC. This might be help for clinicians to estimate the prognosis of ESCC.

Keywords: Esophageal squamous carcinoma (ESCC), single-nucleotide polymorphism, case-control studies, prognosis

Introduction

Esophageal carcinoma (EC) is a world serious malignant neoplasm with regards to the high morbidity and mortality. The two major histologic types of esophageal cancer are squamous cell carcinoma (SCC), which accounts for over 90% cases in Iran, Northern China, India, and Southern Africa and adenocarcinoma (AC), which increased rapidly from mid-1970s and became the predominance in Western country especially in white males in the United States [1-3]. Multiple factors that contribute to esophageal adenocarcinoma (EAC) include low intake of fruits and vegetables, obesity, decreasing of *H. pylori* infection and gastroesophageal reflux disease (GRED) while esophageal squamous cell carcinoma (ESCC) may result from tobacco smoking and alcohol consumption [4-9]. Other potential factors that contribute to ESCC include alendronate use, human papillomaviruses (HPV) and achalasia, which remain controversial [10-12].

With an estimate of 402,000 deaths worldwide in 2012, EC had become the sixth most common cause of cancer-related death and in China and ESCC accounted for 23% of cancer mortality [1, 13, 14]. Although the overall 5-year survival rate remains less than 25% [15], some patients can live for ten years or even longer. This indicates that genes may play an important role in the prognosis of esophageal cancer and several genes, for example, epidermal growth factor receptors (EGFR), *P*53, Bcl-2, cyclooxygenase-2 (Cox-2) et al. have been identified to be predictive/protective molecular markers in patients of esophageal carcinoma [16].

Thanks to the development of sequencing technology, newly genetic markers especially single nucleotide polymorphisms (SNPs) get widely

Table 1. Basic demographic and clinical information

Characteristics	Num (Frequent)	Death	MST (month)	*P*
Total	152	67 (44.1%)	39.45	
Age				
≤60	89 (58.6%)	35 (39.3%)	40.99	0.191
>60	63 (41.4%)	32 (50.8%)	37.28	
Sex				
Male	122 (80.3%)	54 (44.3%)	39.38	0.966
Female	30 (19.7%)	13 (43.3%)	38.57	
Smoking				
Never	68 (44.7%)	33 (48.5%)	37.37	0.333
Ever	82 (53.9%)	34 (41.5%)	40.81	
Unknown	2 (1.3%)			
Location				
Upper/Mid	111 (73.0%)	45 (40.5%)	41.01	0.045
Low	39 (25.7%)	22 (56.4%)	34.23	
Unknown	2 (1.3%)	-		
Tumor differentiation				
Well/Moderate	127 (83.6%)	55 (43.3%)	39.79	0.618
Poor	23 (15.1%)	11 (47.8%)	36.64	
Unknown	2 (1.3%)	-		
TNM				
T				
1-2	48 (31.6%)	14 (29.2%)	45.02	0.013
3	104 (68.4%)	53 (51.0%)	36.89	
N				
Positive	60 (39.5%)	38 (63.3%)	31.12	<0.001
Negative	92 (60.5%)	29 (31.5%)	44.66	
Stage				
I-II	96 (63.2%)	30 (31.2%)	44.57	<0.001
III-IV	55 (36.2%)	37 (67.3%)	29.92	
Unknown	1 (0.7%)	-		
Surgical margin				
Positive	25 (16.4%)	10 (40.0%)	38.16	0.94
Negative	127 (83.6%)	57 (44.9%)	39.72	
Tumor size				
<3 cm	40 (26.3%)	15 (37.5%)	43.31	0.23
≥3 cm	112 (73.7%)	51 (46.4%)	37.94	

applied for inheritance study. In spite that numerous genome wide association analyses (GWAS) of ESCC have been performed, they focused on the tumor occurrence but not prognosis [17-20]. To study the gene effects in the prognosis of ESCC, we performed an observation study in Xi'an Han People based on cohort-study and found three SNPs that may be associated with the prognosis of ESCC.

Materials and methods

Study subjects

A total of 164EC patients were involved in the Yulin First Hospital from 2014 to 2015 and 12 were excluded for various reasons. Six of which were pathologically confirmed as EAC and the remaining six could not be followed up for the wrong information they had left to the hospital. Finally, 152 patients who were pathologically verified as ESCC and underwent esophagectomy were recruited in the study.

Data collecting

Possible clinical factors that may influence the prognosis such as the tumor site, differentiation, TNM stage were collected from the medical record and the main follow-up index was survival state. Other basic demographic characteristics, like age, sex and tobacco smoking were also taken into consideration. Blood samples were collected to extract deoxyribose nucleic acid (DNA) after an informed consent was signed. Survival time was calculated from the date that the esophagectomy was operated to the last follow-up, 2015. Due to time constrains and less than half of the follow-up died at the endpoint, we used the mean survival time (MST) instead of median survival time as the main index to evaluate the prognosis.

Genetic analysis

We selected the candidate SNPs based on the previous published articles that demonstrated SNP polymorphisms which associated with the prognosis of ESCC. Only those SNPs whose minor allele frequency (MAF) >5% in the HapMap CHB population were taken into consideration and finally, a total of 76 SNPs were embraced in the studies. DNA extracted and concentrated were measured by the use of GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xian city, China) and spec-

Table 2. Association between three SNPs and prognosis

SNP	Number	Death	MST (months)	log-rank c^2	log-rank p	Hazard Ratio	95% CI
rs12413624							
TT	46	22	38.07			1	
AT	75	27	42.89	6.87	0.032	0.73	0.41-1.31
AA	31	18	32.57			1.65	0.86-3.19
rs9543325							
TT	47	13	44.78			1	
CT	65	31	39.06	7.61	0.022	1.81	0.93-3.53
CC	35	20	33.35			2.13	1.05-4.34
rs225190							
AA	82	39	37.95			1	
AG	41	14	43.03	6.10	0.047	0.61	0.33-1.14
GG	15	9	28.43			1.58	0.73-3.43

trometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA), respectively. We used the manufacturer of Sequenom MassARRAY RS1000 to genotype SNPs and Sequenom Typer 4.0 Software was applied to analyze the data.

Statistical methods

Microsoft Excel and SPSS 16.0 statistical packages (SPSS, Chicago, IL) were the main software we used to perform statistical analysis. Association between SNP polymorphisms and other possible factors were estimated by the use of Kaplan-Meier method, which assessed by the log-rank test. In order to further evaluate the prognosis of EC, cox proportional hazards regression were performed to eliminate the confounders. Factors whose P<0.2 in the Kaplan-Meier were introduced to the cox's regression models and the final models were calculated using backward stepwise based on Maximum Likelihood Estimate (MLE). Only P<0.05 in the cox regression was considered to be statistical significant.

Results

After patients strictly selection with inclusion and exclusion criteria, 152 ESCC patients from 2014 to 2015 in the first affiliated hospital of Yulin First Hospital were finally included in the study and 122 (80.3%) were males. All patients obtained the revisit, the revisit time are shortest for 1 month and longest for 54 months. Because of the short follow time and less than half of the patients died, we choose mean survival time (MST) instead median survival time.

The basic information of patients is listed in **Table 1**. The number of patients that older than 60 was 89 (58.6%) and 68 (44.7%) patients had never smoked. The TNM stage was: stage I-II 96 (63.2%), stage III-IV 55 (36.2%). Different locations of tumor were also taken into account and 111 (73.0%) patients located in the upper or middle. The numbers of patients whose tumor size less than 3 cm and positive surgical margin were 40 (26.3%) and 25 (16.4%), respectively. At the end of the follow-up visit, 67 patients died and the MST was 39.45 months. No significant results were found in the selected demographic and clinical characteristics such as sex, tobacco smocking tumor differentiation, tumor size and surgical margin. Only the tumor location and TNM stage were found significant associated with the prognosis of ESCC.

We detected 76 SNPs using log-rank test and found 3 SNPs (rs12413624, *P*=0.032; rs9543325, *P*=0.022; rs225190, *P*=0.047) were associated with the prognosis of ESCC. As listed in **Table 2**, genotype "AA" in rs12413624 (MST=32.57) was observed to be the worst compared to genotypes "TT" (MST=38.07) and "AT" (MST=42.59). Moreover, genotype "CC" (MST=33.35) and genotype "GG" (28.43) were found to be the worst in rs9543325 and rs225190 respectively. The survival time of all genotypes were clearly showed in **Figure 1**.

Since the rs12413624, rs9543325 and rs225190 may be associated with the prognosis of ESCC, univariate analysis was performed in these three groups. We found that the age, tumor location and differentiation and TNM stages may affect the prognosis. All the above factors were included in multivariable cox regression analysis and the result was shown in **Table 3**. We finally found that not only the gene factors but also the age and TNM stage were

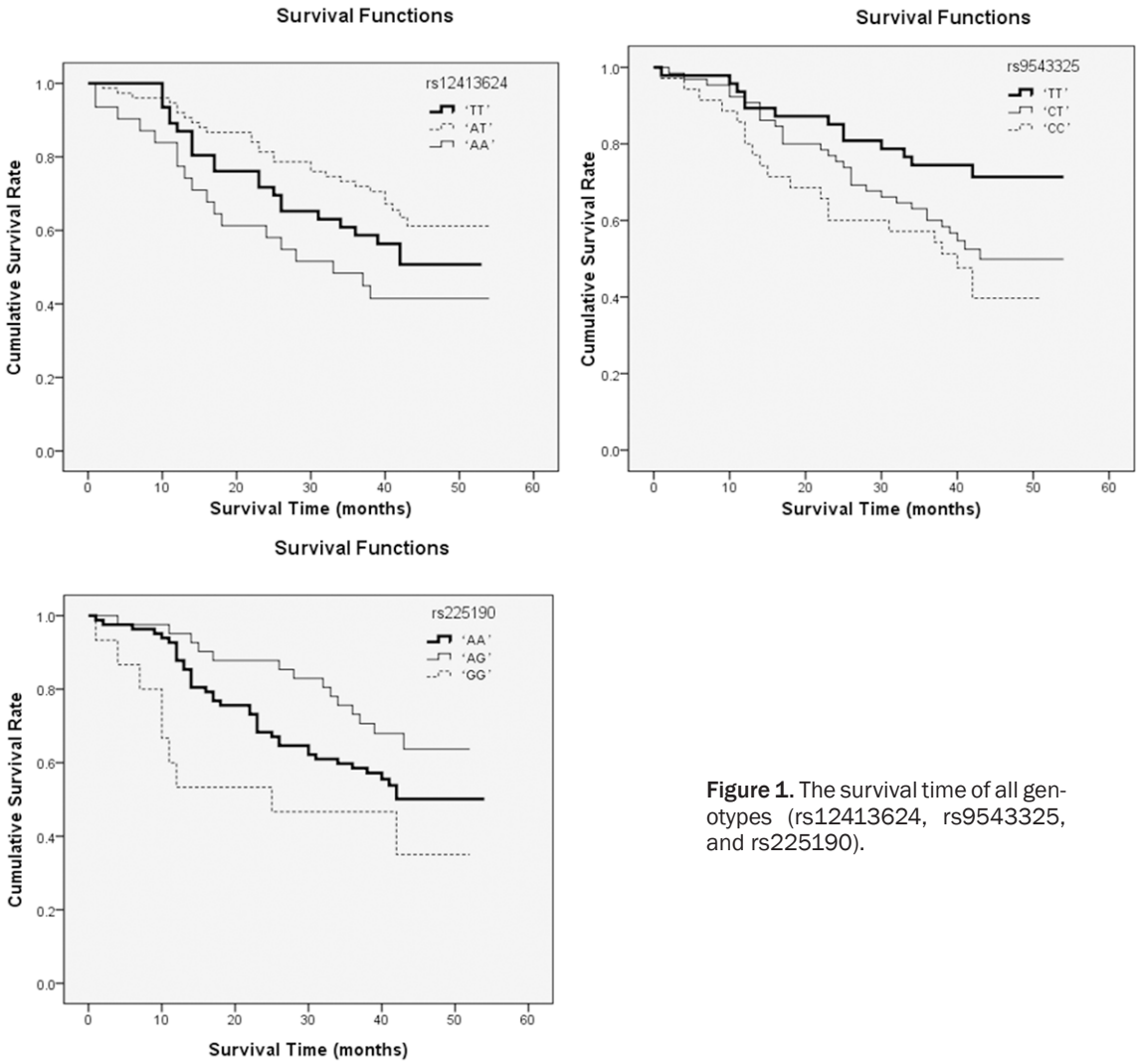

Figure 1. The survival time of all genotypes (rs12413624, rs9543325, and rs225190).

also associated with the prognosis of ESCC. The patients with higher TNM stage (rs12413624: HR=4.53, P=0.01; rs9543325: HR=2.67, P=0.01; rs225190: HR=2.87, P=0.01) and older than 60 (rs12413624: HR=1.87, P=0.02; rs9543325: HR=1.55, P=0.09; rs225190: HR=1.64, P=0.06) had worse prognosis compared to the lower TNM stage and younger.

Discussion

It is well known that there are multiple prognostic factors for ESCC include age, sex, tobacco smoking, alcohol consumption, tumor location, differentiation and TNM stage. But these may be hard to accurately estimate the prognosis while some germline genetic polymorphisms may be a useful marker to predict the prognosis in the next future.

In the current study, we detected 76 SNPs which were reported to be associated with other gastrointestinal tumors and identified three SNPs (rs12413624, rs9543325, and rs225190) associated with the prognosis of ESCC. To the best of our knowledge, this is the first study reporting the association between the three SNPs and prognosis of ESCC. The SNP rs12413624, located downstream of *PRLHR* at 10q26.11, has previously been reported to be associated with pancreatic cancer by Wu, C et al. [21] but showed a negative result in Campa's experiment [22]. The SNP rs9543325, located in a nongenic region of chromosome 13q22 and were identified to be associated with pancreatic cancer [23-25]. A novel amplification of 13q22 may affect the expression of Kruppel-like factor 12 (KLF12) that regulate cell growth and transformation

Table 3. Cox proportional hazard regression

	rs12413624				rs9543325				rs225190			
	HR	95% CI		*P*	HR	95% CI		*P*	HR	95% CI		*P*
		Lower	Upper			Lower	Upper			Lower	Upper	
Age												
≤60	1.00				1.00							
>60	1.87	1.12	3.11	0.02	1.55	0.92	2.62	0.09	1.64	0.98	2.73	0.06
Location												
Low	1.00				1.00							
Upper & mid	0.85	0.50	1.46	0.57	0.75	0.44	1.30	0.75	0.86	0.49	1.50	0.59
Differentiation												
Well & moderate	1.00				1.00							
Poor	1.27	0.67	2.41	0.40	1.36	0.72	2.58	0.35	1.58	0.83	3.04	0.17
TNM												
I-II	1.00				1.00							
III-IV	4.53	0.75	27.48	0.01	2.67	1.52	4.69	0.01	2.87	1.64	4.86	0.01
Genotype[a]												
0	1.00				1.00							
1	0.73	0.41	1.31	0.29	1.77	0.92	3.43	0.09	0.61	0.33	1.13	0.12
2	1.65	0.86	3.19	0.14	2.12	1.04	4.31	0.04	1.58	0.73	3.43	0.24

[a]Different genotypes: rs12413624: 0-TT; 1-AT; 2-AA. rs9543325: 0-TT, 1-CT, 2-CC. rs225190: 0-AA, 1-AG, 2-GG.

[26]. The last SNP rs225190, located in the intro region of *MYO1D*, was also associated with pancreatic cancer [27].

Further analysis showed that genotype "TT" has the longest survival time compared to the left genotypes "CT" and "CC" in rs9543325. It suggested that the allele "C" may contribute to a worse prognosis compared to the allele "T". But it is genotype "AT" not "AA" or "TT" in rs12413624 had the longest survival time. We could not explain whether "A" or "T" contributes to a better prognosis of ESCC but some special reaction must happen when the genotype "AA" and "TT" mutate into "AT" and this need more research. Like rs12413624, the genotype which had longest survival time in rs225190 is "AG".

In addition, clinical factors that may affect the prognosis of ESCC have been taken into consideration. After four steps based on MLE, we identified the age and TNM stage remain associated with the prognosis. The TNM stage which developed by the American Joint Committee on Cancer (AJCC) is widely accepted to estimate the prognosis in clinical and in our study, TNM stage considered to be the most influencing factor.

Actually, there are some limitations in our study that should be considered. First, the sample size of our study was relatively small compared to other similar articles [28, 29]. Second, the follow-time was relatively short and part of the subgroups' mortality rate is less than half. It is a pity that we had to use mean survival time instead of median survival time. Third, we didn't research how the SNPs' mutation affects the patients and then influence the prognosis.

In conclusion, this cohort-study detected 76 SNPs from previous published GWAS especially the gastrointestinal cancer. At last, 3 SNPs (rs12413624, rs9543325, and rs225190) which had been considered to be associated with pancreatic cancer were identified to be associated with the prognosis of ESCC. Although we are the first to report the 3 SNPs associated with the prognosis of ESCC, more work still need to do to clarify the mechanisms of how the SNPs affect the prognosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Peng Zhang, Department of Pathology, Yulin First Hospital, 93 Yuxi Road, High-Tech Zones, Yulin 718000, Shaanxi, China. Tel: +86 29 85323858; Fax: +86 29 85323858; E-mail: zhangpengylin@163.com; Dr. Tianbo Jin, School of Life Sciences, Northwest University, #229 North Taibai Road, Xi'an 710069, Shaanxi, China; National Engineering Research Center for Miniaturized Detection Systems, #229 North Taibai Road, Xi'an 710069, Shaanxi, China. Tel: +86-29-88303800; Fax: +86-29-88303800; E-mail: jintianbo@gmail.com

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