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Polymorphisms of insulin-like growth factor binding protein-3 as a predictor for risk and patient survival in esophageal squamous cell carcinoma

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ABSTRACT

Background and purpose: Genetic single nucleotide polymorphisms (SNP) play a critical role in the development of esophageal squamous cell carcinoma (ESCC). The aim of this study is to investigate the associations between insulin-like growth factor binding protein-3 (*IGFBP-3*) gene polymorphisms and ESCC patients risk and survival after definitive chemoradiotherapy (CRT).
Materials and methods: We undertook a case-control study to analyze two IGFBP-3 polymorphisms (rs2854744 A > C and rs2854746 G > C) in an Han Chinese population, by extraction of genomic DNA from the peripheral blood of 110 ESCC patients treated with CRT and 128 control participants, and performed IGFBP-3 genotyping using DNA sequencing.
Results: The obtained results indicated that overall, no statistically significant association was observed in rs2854746 G > C. However, rs2854744 A > C genotype was at increased risk of ESCCs ($P = 0.032$; odds ratio (OR) = 1.201, CI 95%:1.014–1.423). Moreover, rs2854744 A > C genotype ESCCs were more significantly common in patients with tumor size of >6 cm than A allele ESCC and in cases of lower T stage. Furthermore, ESCC patients with rs2854744CC genotype have the poorer CRT response and shorter survival time than GG + GC genotype ESCC.
Conclusions: In conclusion, polymorphism in IGFBP-3 rs2854744 A > C might be a potential predictor of ESCC risk and patient survival. Nevertheless, further investigation with a larger sample size is needed to support our results.

1. Introduction

Esophageal squamous cell carcinoma (ESCC) is an aggressive human cancer with poor prognosis worldwide [1]. Although, either preoperative chemotherapy or combined chemoradiotherapy (CRT) in association with surgery improved ESCC patients' prognosis, 5-year overall survival rate remains less than 30% [2,3]. Smoking, alcohol drinking, micronutrient deficiency and dietary carcinogen exposure might be main environmental risk factors of this malignant disease [4]. However, accumulating evidence suggests that genetic polymorphisms, such as single nucleotide polymorphisms (SNPs), play an essential role in ESCC development [5,6]. Therefore, discovery of novel biologically functional and risk associated SNPs as ESCC biomarkers might be a potentially valuable path toward clarifying ESCC genetics.

Insulin-like growth factor binding protein-3 (IGFBP-3), a major carrier protein for insulin-like growth factors (IGF)-I or IGF-II in circulation [7], which has been shown to inhibit cell proliferation and activate pro-apoptosis in breast, lung and prostate cancer cells [8]. In our previous study, we observed that the diminished IGFBP-3 expression may be a risk factor for advanced clinicopathologic classifications and poor survival of ESCC patients [9]. Takaoka et al. have also revealed that IGFBP-3 expression is inversely correlated to ESCC in contrast to normal controls, which indicated a role of IGFBP-3 in esophageal malignant progression [10].

The gene encoding *IGFBP-3* locates at 7p14-p12 [11]. In previous studies, two SNPs have been identified as influencing the

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circulating IGFBP-3 levels. One SNP is located at rs2854744 (−202 A > C), a transcription start site, which is supposed to affect the promoter activity [12]. Deal and colleagues observed that circulating IGFBP-3 levels were higher when subjects possessed at least one A allele, and suggested that the circulating IGFBP-3 levels might be regulated by the −202A > C polymorphism [12]. The other SNP is a non-synonymous substitution, rs2854746 (Gly32Ala G > C), a site for high affinity binding of IGF1 [13]. The presence of the variant 32Ala allele was inversely associated with IGFBP-3 levels [14]. Thus, in this study, in order to clarify association between IGFBP-3 rs2854744 (A > C), rs2854746 (G > C) SNP and ESCC risks, we have performed a hospital-based case-control study in Han Chinese population.

2. Materials and methods

2.1. Patients

A total of 110 ESCC patients and 128 healthy controls were qualified in this hospital-based case-control study. All patients treated with CRT were obtained from the Cancer Center, Sun Yat-Sen University, Guangzhou, China, between 2002 and 2008. All samples were collected before any treatment. Histology was determined according to the criteria of the World Health Organization. The study protocol was approved by the Ethics Committee of the Cancer Center, Sun Yat-Sen University in accordance with the Declaration of Helsinki (2000). Tumor stage was assessed according to the AJCC staging system (6th edition). Written informed consent was obtained from all participants.

2.2. Chemoradiotherapy

After completion of diagnose, chemotherapy with PF (cisplatin/5-fluorouracil) regimen and radiotherapy were started concurrently. Cisplatin was administered at a dose of 80 mg/m^2 as an i.v. drip on day 1; 5-fluorouracil 3 g/m^2 was administered as a continuous i.v. infusion for 48 h on days 1–2. Two cycles of chemotherapy were done during radiotherapy at 4-week intervals. External beam radiotherapy was performed by 6–10 MV X-rays. Two dimensional or three-dimensional treatment plans using computed tomography (CT) scans were done. A total radiation dose of 60–70 Gy (1.8–2 Gy/fraction, 5 days a week) was delivered with 3-field technique, and the treatment field was reduced after 40–46 Gy.

2.3. DNA extraction and genotyping

According to the manufacturer's instructions, Genomic DNA from the whole blood cells was extracted using a QIAamp Blood kit (Qiagen, Hilden, Germany). DNA concentration and purity of each sample were measured by ultraviolet spectrophotometer (Eppendorf, Hamburg, Germany). DNA samples were routinely stored at −20 °C. Analysis of *IGFBP-3* SNPs (rs2854744, rs2854746) was performed using multiplex polymerase chain reaction (PCR) with an ABI premix (Applied Biosystems, USA). In each 25 μl reaction, 1 μl genomic DNA (100ng/μl) was amplified by 1.25U Taq DNA polymerase (Takara, Dalian, China) with 2 μl of 2.5 mM dNTPs and 0.5 μl of each primer. The primers for PCR and conditions were performed as previously described [12,13]. PCR products were analyzed on a 3% ethidium bromide added agarose gel, and photographs were taken under ultraviolet light transilluminator. Subsequently, PCR product was sequenced in an ABI PRISM 3100 sequencer using BigDye Terminator v3.1Cycle Sequencing method (Applied Biosystems, USA) as recommended by the manufacturer. Candidate SNP regions were detected and typed with the aid of DNA Star Software (DNASTAR, Madison, WI, USA).

2.4. Statistical analysis

Statistical calculations were performed using the SPSS Statistics 13.0 (SPSS Inc., Chicago, Ill). Frequency and susceptibility to ESCC associated with each mutation were compared using the χ^2 test. The P values obtained were 2-tailed, and the association of significance was assumed to be $P < 0.05$. Hardy–Weinberg equilibrium (HWE) was checked for IGFBP-3 rs2854744 (A > C) and rs2854746 (G > C) variants in ESCC and control subjects by Fisher's exact test. $P > 0.05$ was considered not to deviate from HWE. The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression. Complete response (CR) was defined as the complete disappearance of clinically detectable tumor masses. Survival curves were plotted using Kaplan–Meier survival analysis and compared by log-rank test. Survival time was calculated from the date of ESCC diagnosis to the date of death for any cause. In all cases, differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of subjects

This study comprised 110 ESCC patients and 128 controls. All the cases and controls were selected from the general Han Chinese population of China. Table 1 shows the main characteristics of case-control populations. The age, gender, alcohol consumption, smoking habits and family history of cancer in ESCC patients and controls are not statistically different. The cases and controls were well matched by age (mean ± SD, 58.33 ± 2.21 years in cases and 59.82 ± 3.27 years in controls) and gender (the same proportion for males and females), which suggests that frequency matching was adequate.

3.2. IGFBP-3 gene rs2854744 (A > C) and rs2854746 (G > C) polymorphisms in ESCC

The gene polymorphisms of IGFBP-3 rs2854744 (A > C) and rs2854746 (G > C) were successfully amplified in all of ESCC

Table 1
General characteristics for the ESCC cases and control population.

	No. of cases (%) $N = 110$	No. of controls (%) $N = 128$	P value[c]
Age [a,b]			
≤55	60(54.5)	69(53.9)	0.921
>55	50(45.5)	59(46.1)	
Sex			
Female	90(81.8)	101(78.9)	0.574
Male	20(18.2)	27(21.1)	
Alcohol drinking			
No	76(69.1)	89(69.5)	0.941
Yes	34(30.9)	39(30.5)	
Cigarette smoking			
No	63(57.3)	78(60.9)	0.566
Yes	47(42.7)	50(39.1)	
Family history of cancer			
No	98(89.1)	113(88.3)	0.844
Yes	12(10.9)	15(11.7)	

[a] Age of diagnosis for cases.
[b] Age of control population at the time of diagnosis for the matched case.
[c] P value obtained by χ^2 (cases *vs.*control group).